A Comparison of Molecular Properties of Hepatic Triglyceride Lipase and Lipoprotein Lipase from Human Post-heparin Plasma*  

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Hepatic triglyceride lipase was isolated from human post-heparin plasma by the method of Ehnholm et al. using modifications which increased the specific activity 12-fold to approximately 3,000 μmol of free fatty acid/h/mg of protein. Lipoprotein lipase with similar specific activity was prepared from the same plasma samples using heparin and concanavalin A affinity chromatography. The molecular weight of hepatic triglyceride lipase (69,000) was slightly greater than that of lipoprotein lipase (67,000) as determined by polyacrylamide electrophoresis in sodium dodecyl sulfate-containing buffers. These proteins had identical amino acid compositions, terminal amino acid residues, and tryptic peptide maps. However, the differences previously described regarding optima of pH and ionic strength and the requirement for apolipoprotein CII (only for lipoprotein lipase) were maintained in the highly purified state. It was found that both proteins contain approximately 8% carbohydrate.

Antisera prepared in goats selectively precipitated each activity. Other antisera prepared in chickens reacted with both enzymes, suggesting a common antigenic determinant.

Hepatic triglyceride lipase from human post-heparin plasma can be separated from lipoprotein lipase by affinity chromatography using heparin covalently linked with Sepharose (1–3). Several different techniques have recently been developed for the selective measurement of these enzymes in plasma, including the use of conditions that selectively inhibit LPL activity (4, 5); the precipitation of one of the enzymes with specific antibodies (6, 7), or separation based on the differences in their affinity for heparin (8, 9). When blood plasma is analyzed after intravenous heparin injection the H-TGL is usually detectable with activity comparable to LPL.

There is strong evidence that LPL is located at the luminal surface of the vascular endothelial cells in mammals and birds, since it rapidly appears in tissues perfused with media containing heparin (10, 11). Histochemical evidence suggests that the lipolysis of adherent chylomera occurs at this location (12, 13). Moreover, intravenous injection of antisera specific for LPL results in complete inhibition of the endogenous removal of very low density lipoproteins (14). However, the importance of the enzyme originating from the liver for the intravascular catabolism of triglyceride-rich lipoproteins has not been clearly demonstrated.

Although specific receptor sites for LPL have been postulated, neither the molecular nature of the binding of the enzyme to vascular endothelium nor the mechanism of its release by heparin is known. Less information is available on the subcellular localization of the H-TGL. However, the membrane fraction of rat liver homogenates contains triglyceride lipase activity which may be the source of the activity in post-heparin plasma (15). Isolation of II-TGL has been reported previously (1) and these methods have been modified to provide larger quantities of enzyme with improved specific activity. LPL has also been highly purified from the same sources allowing comparison of the molecular properties of these two enzyme preparations.

MATERIALS AND METHODS

Source of Enzymes

Healthy young men and women in the age range of 21 to 30 were recruited from university personnel and students. The subjects were nonobese with stable weight and no dietary restrictions. After a 12-h overnight fast, a group of four volunteers received an intravenous injection of 60 units of sodium heparin (5000 units/ml, Riker/kg of body weight). Ten minutes later 500 ml of venous blood were collected from each individual into ice-cold IR-1 Fenwal plastic blood pack units (containing no anticoagulant).

Purification of Hepatic Triglyceride Lipase

Plasma Preparation—The plasma was separated from the blood cells by centrifugation for 30 min at 2,000 rpm (4°) in standard 600-ml plastic bags used for collection. The plasma from the subjects was pooled (about 1,200 ml) and centrifuged in a Sorvall RC-2B centrifuge for 30 min at 10,000 rpm (4°) to remove remaining platelets and cellular components. The plasma was then mixed with an equal volume of a buffer containing 0.4 M NaCl, 0.005 M sodium barbital, pH 7.0 (Buffer 1).

Step I—This mixture was then applied at a flow rate of 300 ml/h to a column (6 × 5 cm) of Sepharose covalently linked to heparin.
triolein (5 mCi of \( ^{14} \)C/pmol), 4 mg of gum arabic, 5 mg of albumin mixed with unlabeled triolein (Sigma) to provide the specific activity 10 ml of the scintillation solution, consisting of 15 ml of acetic acid, 1 ml of an equal volume of 0.005 M sodium barbital, pH 7.0 (Buffer 4). The enzyme solution was then eluted with 250 ml (200 ml/h) of the latter buffer containing 1 M \( \alpha \)-methyl-d-glucopyranoside. This eluate was dialyzed for 16 h against 15 liters of a glycine solution (1 g/dl) followed by two changes after 4 and 8 h. The preparation was then concentrated to 200 ml using the Amicon ultrafiltration system with a UM-40 filter.

**Purification of Lipoprotein Lipase**

**Step I** — The LPL obtained as described above in Step I was used for further purification.

**Step II** — A concanavalin A-Sepharose column (6 x 15 cm) was equilibrated as described for H-TGL alone (Step II). After direct application of the eluate from the heparin-Sepharose column containing lipoprotein lipase in Buffer 3, the column was washed with 350 ml of Buffer 4 at a flow rate of 200 ml/h. The enzyme was then eluted with 250 ml of Buffer 4 (flow rate 100 ml/h) containing 1 M \( \alpha \)-methyl-d-glucopyranoside.

**Step III** — The enzyme solution contained in 250 ml from Step II was dialyzed for 1 h against 12 liters of 0.005 M sodium barbital, pH 7.0, with one change after 30 min. The dialysate was then mixed with an equal volume of 0.005 M sodium barbital, pH 7.0. The total volume was applied to a second heparin-Sepharose column (1 x 2.5 cm) which had been equilibrated as in Step I. The column was washed with 200 ml of Buffer 1 and 100 ml of Buffer 2. The enzyme was eluted at 0.005 M sodium barbital (pH 7.0) using a linear NaCl gradient, ranging from 0.75 to 1.85 M at a flow rate of 100 ml/h and in a total volume of 600 ml (Fig. 3). NaCl concentrations were determined by comparative conductivity measurements with standard solutions, using a Radiometer model CD M 2 conductivity meter. The fractions containing enzyme activity were combined, dialyzed against 15 liters of 0.005 M ammonium bicarbonate with four changes in 72 h, and lyophilized. The enzyme solution was stored under liquid nitrogen.

**Lipoprotein Lipase Assay**

Unless otherwise stated, all assays were conducted as described above. The assay mixture was incubated at 28°C for 30 min (9). Each milliliter of the final incubation mixture contained: 0.2 nmol of Tris/HCl buffer (pH 8.2), 0.15 mmol of NaCl, 2.3 \( \mu \)mol of trypsin (Sigma), 4 mg of gum arabic, 5 mg of albumin and 20 \( \mu \)g of apolipoprotein CII. The enzyme was then diluted with an equal volume of 0.005 M sodium bicarbonate and dialyzed against 15 liters of a glycine solution (1 g/dl) followed by two changes after 4 and 8 h. The preparation was then concentrated to 200 ml using the Amicon ultrafiltration system with a UM-40 filter.

**Hepatic Triglyceride Lipase Assay**

Assays were conducted as described for LPL above. However, each milliliter of the final incubation mixture contained: 0.2 mmol Tris/HCl buffer (pH 8.8), 0.75 mmol of NaCl, 2.2 \( \mu \)g of triolein (5 mCi of \( ^{14} \)C), 4 mg of gum arabic, and 5 mg of albumin. In certain experiments protamine sulfate was added 10 min prior to the enzyme solutions in concentrations described under "Results."

**Polyacrylamide Gel Electrophoresis**

Samples of 50 or 100 \( \mu \)g of the protein were incubated for 20 min at 90°C in 200 \( \mu \)l of a buffer consisting of 1 mm sodium phosphate (pH 7.1), 1% sodium dodecyl sulfate, 1% mercaptoethanol, and 0.001% bromphenol blue. Solid sucrose was then added to the samples and disc gel electrophoresis was carried out as previously described (18) using tubes (0.8 x 10 cm) pretreated with Photo-flo 600 (Eastman Chemical). The gel was prepared with 10% polyacrylamide and contained 0.1% sodium dodecyl sulfate. A constant current of 8 mA/gel was applied at room temperature until the marker dye moved a predetermined distance within the tube. The gels were then fixed and stained overnight in 12.5% trichloroacetic acid, containing 0.025% C-250 Coomassie brilliant blue as stain. They were then destained with 5% acetic acid. For molecular weight determinations human transferrin, albumin, ovalbumin, chymotrypsinogen A, and H- and L-chain of y-globulin were used as standards (Schwarz/Mann). Some gels were stained with the periodic acid-Schiff reagent (19).

**Gel Filtration**

The molecular weight of the H-TGL enzyme was also determined by gel chromatography with a column of Bio-Gel P-100 (40 x 2.5 cm) using Buffer 4 together with 1 M \( \alpha \)-methyl-d-glucopyranoside as the eluant. The enzyme preparation (5 ml) was that obtained in Step II. Fractions of 4 ml were collected at a rate of 4 ml/h, and subsequently assayed for protein concentration (20) and enzyme activity. The void volume was determined with blue dextran 2000 (Pharmacia). Myoglobin, chymotrypsinogen A, ovalbumin, albumin, and human transferrin, as obtained from Schwarz/Mann, were used as standard markers.

**Amino Acid Analysis**

Lyophilized protein samples containing 10 to 20 nmol were dissolved in 1 ml of 6 N HCl and hydrolyzed under vacuum at 110°C for 24, 48, and 72 h. The amino acids were quantitated with a Beckman model 121 amino acid analyzer fitted with columns containing Beckman AA 15 and PA 35 resins. Experimental values for serine and threonine were corrected by extrapolation of the data to zero time hydrolysis. The values of valine, leucine, and isoleucine, determined after 72 h of hydrolysis, were used for the final calculations of composition. For the determination of tryptophan, samples were hydrolyzed for 22 and 44 h in 1 ml of 3 M methanolic \( p \)-toluenesulfonic acid containing 0.2% 2-12-aminoethylindole at 110°C in vacuo. After neutralization with 1 ml of 1.0 M NaOH the solution was filtered through a Swinney adapter, fitted with a GS-0.22 micron Millipore filter (21). Chromatography was performed on a 20-cm column of PA 35 resin.

**Formation of Dansyl Amino Acids**

Samples containing 5 to 10 nmol of protein were reacted with 10 \( \mu \)l of dansyl chloride (Pierce) at a concentration of 2.4 mg/ml in acetonitrile and distilled water (1:1, v/v) at pH 10 for 1 h at 30°C. After evaporation samples were hydrolyzed in 6 N HCl for 18 h at 110°C: the solutions were lyophilized, reconstituted in 5 \( \mu \)l of 50% pyridine, and spotted on polyamide thin layer plates (Gallard-Schlesinger). The plates were then developed in three solvent systems as described previously (22). The spots were visualized under ultraviolet light and their location compared with standard dansyl-amino acids (Pierce).
**Hepatic Triglyceride Lipase and Lipoprotein Lipase**

**Formation of Phenylthiohydantoin**

Samples of protein (5 to 10 nmol) were treated with phenylisothiocyanate (Beckman) and the phenylthiohydantoin derivatives were formed following the procedure described in the Beckman sequenator manual. They were then chromatographed on polyamide thin layer plates (23). Samples and standards were inspected under shortwave ultraviolet illumination.

**Carboxypeptidase A Digestion**

Approximately 10 nmol of protein together with norleucine as an internal standard were treated with carboxypeptidase A (Sigma) using a protein-to-enzyme ratio (by weight) of 150:1 at 27°C in 0.2 M NaHCO₃. Aliquots were taken at different time intervals, frozen immediately in liquid nitrogen, and lyophilized. After precipitation of the protein with 10% trichloroacetic acid, v/v, the samples were filtered through a capillary pipette filled with glass wool and subjected to amino acid analysis as described above.

**Hydrazinolysis**

After drying over P₂O₅ for 24 h in a desiccator, samples containing approximately 5 nmol of protein were held for 22 h at 80°C in 0.2 ml of anhydrous hydrazine (Matheson, Coleman and Bell). The samples were then dried in a stream of nitrogen and dissolved in 1 ml of water. The hydrazides were separated from the amino acids in capillary columns of Amberlite CG-50. The solution containing the liberated amino acids were then lyophilized and analyzed on an amino acid analyzer (24).

**Peptide Mapping**

The purified lipases (0.5 mg) were dissolved in 1 ml of a 1% (w/v) ammonium bicarbonate solution. Ten micrograms of trypsin (Serva) were added initially, and again after 2 and 4 h. After incubation at 50°C for 24 h, the samples were lyophilized, dissolved in 50 μl of a pyridine/acetic acid/H₂O buffer (5:5:90, v/v), pH 4.7, and spotted on filter paper. Electrophoresis was carried out for 4 h in the same buffer system using a Savant instrument (model HVS-8036) at 2000 V. The filter papers were then dried. Chromatography for 24 h in butanol/acetone/H₂O buffer (50:30:40, v/v) solvent system ensured. Filter papers were again dried and stained with 0.02% ninhydrin (Serva) in acetic acid/pyrindine/acetone (11:10, v/v).

**Carbohydrate Analysis**

The carbohydrate composition of the enzymes was determined by gas-liquid chromatography of the trimethylsilyl ethers (26). Protein samples were lyophilized together with arabinol and methyloxime for 24 h at 100°C. The amounts of each sugar were calculated by the method of Clamp et al. (26). Amino sugars were also determined on lyophilized samples hydrolyzed in 4 N HCl at 110°C for 8 h (27). Hydrolysis was also performed in 3 M hydrochloric acid for 24 h at 110°C (21). Chromatography followed on a 10-cm column of PA 35 resin of a Beckman model 121 amino acid analyzer.

- N-Acetylneuraminic acid was detected by the procedure of Warren (28). The presence of N-acetylneuraminic acid was confirmed following hydrolysis in 1 M hydrochloric acid for 24 h at 100°C (27). Hydrolysis was also performed in 3 M hydrochloric acid for 24 h at 110°C (21). Chromatography followed on a 10-cm column of PA 35 resin of a Beckman model 121 amino acid analyzer.

- Other Procedures

**Fluorescence Spectrophotometry**

Excitation and emission spectra of enzyme solutions (200 μg/ml) were recorded on a Hitachi-Porkin-Elmer MFP-2A spectrophotofluorometer at room temperature as described elsewhere (30). The buffer used was 0.2 M KCl, 0.02 M PO₄, pH 6.5.

**Immmunochemical Procedures**

Purified enzyme (500 μg) was suspended in 1 ml of 0.15 M NaCl and after addition of 1 ml of complete Freund's adjuvant (Difco) the mixture was subjected to sonic irradiation for approximately 2 min. Such preparations of both H-TGL and LPL were injected subcutaneously into 1- to 2-year-old goats (200 μg) and 10- to 20-week-old hens (100 μg). After 4 weeks and then at 2-week intervals, the same procedure was repeated with each animal. Antisera from individual goats, each injected with LPL (176) and H-TGL (177) were used in the present studies. These sera were obtained after the fourth injection of enzyme.

The effect of antisera addition on enzyme activity was determined by mixing 20 μl of purified H-TGL or LPL solution with 200 μl of goat sera, prepared by mixing varying proportions of the appropriate antisera with sera from a nonimmunized goat. After incubation for 1 h at 27°C, centrifugation was done in an Eppendorf centrifuge (model 3200) and 50 μl of the supernatant solutions were added to appropriate assay media for LPL or for H-TGL.

**RESULTS**

**Purification of Human Hepatic Triglyceride Lipase**—The H-TGL was eluted from a heparin Sepharose column by 0.75 M NaCl had a specific activity about 2,000-fold higher than that of post-heparin plasma with a yield of about 70% (Fig. 1). The pooled eluate (280 ml) was then directly bound to Concanavalin A-Sepharose. Only traces of enzyme activity did not bind to the column. H-TGL was more effectively displaced from its binding to concanavalin A by α-methyl-D-glucopyranoside than by α-methyl-D-mannopyranoside. The specific activity in the eluate (350 ml) was more than doubled by this step and the recovery was almost 80% of the applied activity.

**FIG. 1. Heparin-Sepharose chromatography of human plasma lipase activity.** Post-heparin human plasma, 2.26 liters after dilution (see text), was applied directly to the column (Step A). The effluent from the column was then collected in 50-ml fraction throughout all subsequent steps and assayed for protein concentration (●) and enzyme activity (■■■■) under conditions optimal for LPL (see text). After washing with 4 liters of 0.4 M NaCl buffer (Step B) the activities were eluted with sequential stepwise increase in the NaCl concentrations of the buffer as shown —— (36). Most of the hepatic triglyceride lipase eluted in the first 350 ml of buffer in Step C (0.75 M NaCl). The lipoprotein lipase activity was contained in 250 ml of Step D buffer (1.5 M NaCl). FFA, free fatty acid.
pl 4.95 was almost 15,000-fold compared with the starting material. The pl of LPL (4.5) differed from all three activities observed with H-TGL. A summary of the purification procedure is given in Table I.

Purification of Human LPL—The elution pattern of the lipolytic activity of human post-heparin plasma from a heparin-Sepharose column is shown in Fig. 1. Over 99% of the protein appeared in the column elute during loading and in the washing phase with Buffer 1 which contained 0.4 M NaCl. The adjustment of the column buffer to 0.4 M NaCl and the plasma after dilution to about 0.3 M NaCl for the loading phase resulted in less binding of undesired proteins and an apparent increased binding efficiency for the enzyme activity. Under these conditions, essentially no activity was found to elute during the loading process. Most importantly, this method gave a marked improvement in the yield of lipoprotein lipase (9).

The pooled eluate containing lipoprotein lipase (1.5 M NaCl) had a specific activity about 2000-fold greater than that of the original plasma with a 55% yield (Table II). At this stage of purification the LPL activity was increased 10- to 20-fold on addition of the activator protein, apo-CII.

The human lipoprotein lipase was found to bind to concanavalin A-Sepharose with only traces of enzyme activity eluting during the loading phase (Fig. 3). Both α-methylglucoside and α-methylmannoside were partially effective in displacing the enzyme from the column; however, the recovery was best with α-methyl-D-glucopyranoside. Of the total lipase activity applied, 98% was eluted in 250 ml of the α-methyl-D-glucopyranoside buffer and the specific activity of the pooled eluate was almost 8,000 times that of the post-heparin plasma (Table II). A major band of \( M_r = 65,000 \) to 70,000 and a few lower molecular weight proteins were found on sodium dodecyl sulfate-polyacrylamide gel electrophoresis of this preparation.

**Table I**

| Purification of human hepatic triglyceride lipase | Volume | Protein Total activity Specific activity | Purification | Yield |
|---|---|---|---|---|
| Human post-heparin plasma | 1,130 | 83,055 | 17,522 | 0.21 | 100 |
| Step I. Heparin-Sepharose chromatography | 250 | 10.4 | 3,972 | 0.46 | 72 |
| Step II. Concanavalin A-Sepharose chromatography | 350 | 10.4 | 3,972 | 0.46 | 54 |
| Step III. Isoelectric focusing | 100 | 1.2 | 3,680 | 3,067 | 21 |

**Table II**

| Purification of human lipoprotein lipase | Volume | Protein Total activity Specific activity | Purification | Yield |
|---|---|---|---|---|
| Human post-heparin plasma | 1,130 | 83,055 | 17,522 | 0.21 | 100 |
| Step I. Heparin-Sepharose chromatography | 250 | 10.4 | 3,972 | 0.46 | 72 |
| Step II. Concanavalin A-Sepharose chromatography | 350 | 10.4 | 3,972 | 0.46 | 54 |
| Step III. Heparin-Sepharose chromatography | 165 | 0.46 | 3,181 | 2,865 | 17.9 |

*Step I is identical with Step I in Table I except the fraction analyzed was that eluting with 1.5 M NaCl in barbital buffer as described in the text and in Fig. 1.
Hepatic Triglyceride Lipase and Lipoprotein Lipase

The latter comigrate with proteins eluting from concanavalin A-Sepharose without addition of other protein material as previously reported (37). The preparation from this step of the purification procedure could be stored at -70° in the 1 M α-methylglucopyranoside buffer for several weeks without major loss of activity.

Application of the enzyme solution to a second heparin-Sepharose column after the concanavalin A-Sepharose chromatography (Fig. 5) required reduction in both the salt and sugar concentrations to achieve binding. Dialysis for 4 h resulted in a 50% loss of enzyme activity, whereas a combination of dialysis for 1 h followed by dilution resulted in only a 20% loss of activity and complete binding to heparin-Sepharose. The enzyme solution eluted during the application of the NaCl gradient had more than a 4-fold increase in specific activity and a final purification of 32,000 times that of the original plasma (Table II). Storage at -70° resulted in approximately 50% survival after several weeks. Lyophilization, however, completely inactivated the purified enzyme. The protein contained less than 0.01% phospholipid, and no cholesterol or triglyceride could be detected by thin layer chromatography.

Protamine Sulfate Sensitivity—Highly purified H-TGL was not affected by protamine sulfate concentrations up to 5 mg/ml in the incubation mixture, whereas LPL was almost completely inactivated at levels above 2 mg/ml. Similar results were obtained when the compound was added directly to the enzyme solutions 10 min before the assay was conducted.

Polyacrylamide Gel Electrophoresis—A single major band was obtained on sodium dodecyl sulfate-polyacrylamide gel electrophoresis after the final stage of purification (Fig. 6) with both H-TGL and LPL. The band from the H-TGL preparation had slightly slower mobility than that of LPL (as shown in Fig. 6) when small quantities of both preparations were loaded together on a single polyacrylamide gel. These bands could be stained with the periodic acid-Schiff reagent after removal of the sodium dodecyl sulfate from the gel. The protein contained less than 0.01% phospholipid, and no cholesterol or triglyceride could be detected by thin layer chromatography.

![Electrophoresis patterns of human lipoprotein lipase in 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate.](image1)

![Polyacrylamide gel electrophoresis pattern of purified H-TGL and LPL.](image2)
mobilities of these bands indicate a molecular weight for human lipoprotein lipase of approximately 67,000, and for the hepatic triglyceride lipase of 69,000. For molecular weight determinations, comparison with marker proteins was performed at five different polyacrylamide concentrations to determine the possibility of aberrant behavior in this system observed with some other glycoproteins (38). No abnormal migration was found in these gels. The polyacrylamide gel electrophoresis pattern was not changed by performic acid oxidation or reduction and alkylation, indicating a single polypeptide chain for the major protein in both H-TGL and LPL.

**Gel Filtration**—LPL activity was found to bind to Sephadex even in the presence of 1 or 2 m NaCl. The apparent binding was much less during gel filtration on polyacrylamide beads (Bio-Gel P-100) in the presence of 1 m NaCl and 1 m α-methyl-D-glucoside. The sugar was found to be necessary for stabilization of the activity. The enzyme activity eluted from the column of Bio-Gel P-100 coincided with a major protein peak (Fig. 5) and gave a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis with the same mobility as the preparation from the second heparin-Sepharose column (Fig. 5). The elution volume corresponded to that expected for a protein of about 67,000 and thus confirmed the measurement made by sodium dodecyl sulfate electrophoresis. In some experiments a small fraction of the activity eluted from the column was found in the void volume. This protein also had the same mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis as the material of 67,000, suggesting partial aggregation of the enzyme protein with maintenance of some activity.

**Amino Acid Composition**—The amino acid composition of H-TGL and of LPL preparations show no significant differences (Table III). Two different hydrolysis procedures have been used for this analysis, each with at least six different protein preparations, i.e. 48 donors. Irrespective of the type of hydrolysis, the values for the two enzymes were indistinguishable. Determinations of aspartic and glutamic acid showed the highest deviations from the mean. The polarity index (39) of 50% shows an equal partition of hydrophilic and hydrophobic residues.

**Terminal Amino Acid Analysis** The NH-terminal amino acid is glycine for both enzymes as determined by dansylation or formation of the phenylthiohydantoin derivatives. The COOH-terminal amino acid is serine for both H-TGL and LPL as determined by digestion of the protein with carboxypeptidase A and by hydrazinolysis.

**Peptide Mapping**—Tryptic digestion of H-TGL and LPL over 24 h led to a complete hydrolysis and solubilization. At least 35 to 40 distinct peptides for each enzyme protein were evident by electrophoresis and chromatography (Fig. 8). In addition, a large amount of material remained near the origin and was poorly resolved. When the two enzymes were digested and the peptides analyzed in parallel, the patterns of staining were indistinguishable (Fig. 8). These results strongly suggest that both preparations contain identical polypeptides.

**Fluorescence Spectrophotometry**—H-TGL and LPL show an excitation maximum at 286 nm typical for tryptophan. Fluorescence intensity is identical for comparable amounts of enzyme proteins, supporting the equivalence of the amount of tryptophan as determined by amino acid analysis. The emission spectrum of LPL has a maximum at 336 nm but there is a small blue shift to 334 nm in the spectrum of H-TGL.

**Carbohydrate Analysis**—Analysis of several preparations of H-TGL and LPL by gas-liquid chromatography revealed the presence of about 2% (w/w) mannose, 2% galactose, and about 3% glucosamine. Approximately 1% sialic acid was also detected. The content of amino sugars was determined to be about 4% following hydrolysis in either 4 N HCl or 3 N-toluene sulfonic acid and quantitation on the amino acid analyzer. The presence of about 1% sialic acid was further confirmed by the thiobarbituric acid assay or by methanolysis in 1 N p-toluene sulfonic acid. Although modest differences between the two enzyme preparations were found, these could be within the error of the analyses. The quantities of glucose found in both enzyme preparations varied over a range of 1 to 7% of the protein mass. Although the values were usually higher for H-TGL it is believed that the presence of most, if not all, of this sugar may be explained by contamination. It has therefore been omitted from the calculations for percentage composition and is not used in correcting the molecular weight of the polypeptide chain. Glucuronic or iduronic acids were not detected by GLC (26). This method would detect the

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**Table III**

| Amino acid composition | Hepatic triglyceride lipase | Lipoprotein lipase |
|------------------------|-----------------------------|-------------------|
| Alanine 69.5 (± 1.2) 38 | 67.1 (± 0.9) 37 | |
| Aspartic acid 82.7 (± 11.4) 45 | 81.4 (± 18.3) 46 | |
| Asparagine 62.6 (± 3.8) 34 | 63.4 (± 4.7) 35 | |
| Glutamic acid 43.4 (± 0.6) 24 | 44.3 (± 1.1) 24 | |

*The molecular weight used was 62,500, the value obtained after correcting for carbohydrate content.
Normal goat serum and antisera prepared against the alter-

volumes of antisera raised against the appropriate enzyme.

However, important modifications have improved the

direction of the arrows as described in the text. -

Following electrophoresis for 4 h, chromatography was performed in

direction of the arrows as described in the text.

FIG. 8. Peptide mapping of purified H-TGL and LPL. After tryp-
tic digestion of the enzymes, 500 μg of each preparation of H-TGL
(bottom) and LPL (top) were applied at the origin as indicated.
Following electrophoresis for 4 h, chromatography was performed in

presence of less than 0.2% (w/w) of these sugars. Thus, there

is no evidence for the presence of heparin in either enzyme

preparation.

Immunoinactivation - The triglyceride hydrolase activity of

H-TGL and LPL were progressively inhibited by increasing

volumes of antisera raised against the appropriate enzyme. Normal goat serum and antisera prepared against the alternate enzyme did not inhibit H-TGL or LPL (Fig. 9).

DISCUSSION

The H-TGL has been previously purified to near homogene-

ity by essentially the same sequence of procedures as reported

here. However, important modifications have improved the

yields and allowed a 12-fold increase in specific activity. Increasing the ionic strength of the plasma before loading onto the heparin-Sepharose column was the most important of these changes. This procedure removed the need for a delipi-
dation step and thereby avoided a very large loss of activity

for both H-TGL and LPL. The introduction of isoelectric

focusing as a preparative procedure added an additional 3-fold

purification to the present result with H-TGL. This technique

also demonstrated heterogeneity within the H-TGL activity,

since at least two and perhaps three isoelectric points were

found for enzyme preparations from several different plasma

pools. Recently, two forms of H-TGL have been separated by

NaCl gradient elution of heparin-Sepharose (40). The nature

of this heterogeneity is yet to be defined.

Chromatography on concanavalin A-Sepharose was most

successful with both enzymes when the column was eluted

with α-methyl-D-glucopyranoside. This is identical with ear-
er findings with the human hepatic lipase (1), but differs

from the chromatography of swine adipose tissue lipoprotein

lipase, with which better recovery was obtained using α-
methyl-D-mannoside (37). With LPL, a second hepatic-Sepha-

rose column was required in part to remove protein contami-
nants which were introduced during the concanavalin A-

Sepharose chromatography step and which may represent
degradation products of the concanavalin A itself. These

products have been eliminated in the purification of chicken

adipose tissue LPL by immuno adsorption with antibodies

prepared against concanavalin A (14). Similar material was

separated from H-TGL by the isoelectric focusing procedure.

The specific activity of the LPL preparation in its most

highly purified form was approximately 3000 μmol/h/mg of

protein. This is comparable to values obtained with LPL from

swine adipose tissue (37) and an LPL from rat plasma (41).

The enzyme obtained from chicken adipose tissue (14) had

approximately twice the activity per mg of protein using

similar assay techniques. With the purified bovine milk LPL

(42), specific activities have been found which are 10-fold

greater than those obtained with the human plasma enzyme.

This is, in part, explained by the assay of the milk LPL under
different conditions, including the use of incubations at 37°
versus those at 28° in the present experiments. It is also clear

that species differences in kinetic characteristics may exist.

The molecular weight of LPL determined by gel filtration is

in agreement with that of the band of protein found on sodium
dodecyl sulfate-polyacrylamide gel electrophoresis (67,000).

This latter technique has given size estimates of lipoprotein

lipase from bovine milk (43), swine adipose tissue (37), chicken

adipose tissue (14) had approximately twice the activity per mg of protein using

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This latter technique has given size estimates of lipoprotein

lipase from bovine milk (43), swine adipose tissue (37), chicken

adipose tissue (14), rat (44) and swine heart (45), and the

hepatic lipase (1) from human plasma that range from 60,000
to 70,000. It also agrees with an earlier report on the human

plasma lipoprotein lipase (46). The highly purified bovine

milk enzyme has recently been studied by sedimentation

equilibrium ultracentrifugation and by gel filtration in the

presence of guanidine HCl. By both techniques, the protein

was estimated to have molecular weight of approximately

50,000 (42).

It has also been reported recently that two forms of apo-CII

activated lipoprotein lipase are released into post-heparin

plasma of the rat (47). One of these has a much lower

molecular weight (37,500) and a higher affinity for triglycer-

ide-rich lipoproteins than the second species of LPL (Mₐ =

69,000). Both enzymes are inhibited by apolipoprotein CI and

apolipoprotein CIII and by high ionic strength or protamine in

the assay media. The so-called low affinity lipase is similar in
size to LPL purified from human plasma and swine adipose tissue (37). The lower molecular weight LPL may originate from muscle, since an enzyme in this size range (34,000) has been purified from rat heart muscle (48). However, the low molecular weight rat enzymes from plasma and heart differ in content of certain amino acids and in the quantity of carbohydrate. The latter LPL contains only 3.3% sugar by weight, whereas the plasma low molecular weight LPL has 6.9%. This difference may reflect incomplete glycosylation of intracellular enzyme from the heart tissue homogenates. The total sugar content of the human post-heparin plasma enzymes is about 8% for H-TGL and for LPL. These values are very similar to the content of 8.3% reported for bovine milk lipoprotein lipase (42). The human enzymes have a content of N-acetylglucosamine which is similar to that of rat plasma (high molecular weight) LPL (47). A neutral sugar analysis on this enzyme has not yet been reported.

The comparative studies of the isolated H-TGL and LPL have revealed two very similar proteins. They both appear to be single polypeptide chains with the same terminal amino acid residues, amino acid composition, and tryptic peptide maps. However, a number of differences have also been noted. LPL has a slightly lower molecular weight, a lower isoelectric point, and a fluorescence emission maximum at a higher wavelength than H-TGL (Table IV). The two activities are also distinguishable by immunological means using antisera prepared to each of these purified proteins. The most substantial distinguishing feature continues to be the requirement of LPL for apolipoprotein CII as a cofactor for hydrolysis of triglycerides. In addition, there are marked differences in the rates of hydrolysis of other substrates (49, 50). The sensitivity to inhibition by changes in ionic strength or addition of protamine sulfate (4) and certain detergents (50) is also similar.

The differences in molecular weight between H-TGL (69,000) and LPL (67,000) from human plasma could be explained within experimental error by differences in sugar content. However, the kinetic differences would seem to require an alteration in the tertiary structure of the polypeptide chain. Such a conformational difference is suggested by the fluorescence spectra, with a lower emission maxima for H-TGL being compatible with the location of one or more tryptophan moieties in a less polar environment. Although sugar differences can explain the chromatographic properties of several proteins (51, 52) including porcine pancreatic lipase A and B (53), distinguishing kinetic features have not been found to accompany this type of structural difference (54). However, the activity of acid phosphatase from human prostatic gland has been altered in both the \( K_m \) and \( V_{max} \) by removal in vitro of neuraminic acid (5), and the stability of the invertase of *Neurospora* is markedly affected by removing the carbohydrate moieties (56). If the carbohydrate moieties are the only structural difference between H-TGL and LPL, this would be a most dramatic example of modification of enzyme activity by glycosylation. In effect tissue-specific iso-enzymes would be the result of the nature of the sugar attachment. In studies using porcine submaxillary gland and porcine liver, differences have been found in the sialyltransferase activities with both an apolipoprotein and ovine submaxillary mucin as acceptor proteins (57). Thus, different glycosyltransferases may exist in different tissues within the same species, providing the basis for organ specificity. Substitutions in the amino acid sequence remain a possibility since a single residue difference has been shown to occur in actin from human platelets versus that from muscle (58). This suggests that certain proteins may have separate independently controlled genes. Other possible mechanisms include interconversion of one enzyme to the other by phosphorylation, sulfation, or even peptide cleavage of a small segment. The presence of identical terminal amino acid residues is against the latter hypothesis and no phosphorus was found on analysis of the whole purified proteins. Analysis for sulfate has not been done.

It seems less likely that two completely different proteins are responsible for the enzymatic activities. This hypothesis could require that each lipase co-chromatograph through all systems used with a single major contaminant which exists in two chromatographically separable forms.

An alternate hypothesis is that one of the two enzymes exist in an active and an inactive form. The inactive form then chromatographs separately on heparin-Sepharose but coincidentally with the other lipase. This would require that the contaminated enzyme have an extremely high specific activity, thereby contributing little polypeptide in the compositional analyses.

Detailed analysis and perhaps chemical alteration of the carbohydrate side chains of H-TGL and LPL will be required to establish the significance of any compositional differences found. The final proof will depend on experiments which convert one enzyme into a form having the chromatographic and kinetic properties of the other. These considerations suggest that the genetic deficiency of lipoprotein lipase as seen in type I hyperlipoproteinemia should now be re-examined. Since H-TGL is present in near-normal levels, an abnormality in a gene other than the one which codes for the enzyme polypeptide chain may underlie this disorder.

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### REFERENCES

1. Ehnholm, C., Shaw, W., Greten, H., and Brown, W. V. (1975) *J. Biol. Chem.* 250, 6756-6761
2. Greten, H., Walter, B., and Brown, W. V. (1972) *FEBS Lett.* 27, 306-310
3. Ehnholm, C., Shaw, W., Brown, W. V., Lengfelder, W., and Greten, H. (1973) in *Atherosclerosis III: Proceedings of the Third International Symposium* (Scheffler, G., and Winzel, A., eds.) pp. 557-560, Springer-Verlag, Berlin
4. Krauss, R. M., Windmüller, H. G., Levy, R. I., and Fredrickson, D. S. (1976) *J. Lipid Res.* 17, 296-298

**Table IV**

| Human post-heparin lipolytic activity | Hepatic triglyceride lipase | Lipoprotein lipase |
|--------------------------------------|-----------------------------|--------------------|
| **Molecular weight**                 | 69,000                      | 67,000             |
| **pl**                               | 4.95                        | 4.5                |
| **Excitation maximum (nm)**          | 289                         | 289                |
| **Emission maximum (nm)**            | 334                         | 336                |
| **NH2-terminal**                     |                             |                    |
| a. Dansyl derivative                 | Glycine                     | Glycine            |
| b. PTH derivative                    | Glycine                     | Glycine            |
| **COOH-terminal**                    |                             |                    |
| a. Carboxypeptidase digestion        | Serine                      | Serine             |
| b. Hydrazinolysis                    | Serine                      | Serine             |
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5. Krauss, R. M., Levy, R. I., and Fredrickson, D. S. (1974) J. Clin. Invest. 54, 1107–1124
6. Ehnholm, C., Huttunen, J. K., Kinnunen, P. J., Mettinen, T. A., and Niskila, E. A. (1975) A. Engl. J. Med. 292, 1314–1317
7. Green, H., Degrella, R., Kelso, G., Ruscher, W. L., DeGennes, J. L., and Gjone, E. (1976) J. Lipid Res. 17, 203–210
8. Ehnholm, C., Shaw, W., Harlan, W., and Brown, W. V. (1973) Circulation 48 (Suppl. IV), 112
9. Boberg, J., Augustin, J., Baginsky, M. L., Tejada, P., and Brown, W. V. (1977) J. Lipid Res. 18, 544–547
10. Ho, S. J., Ho, R. J., and Meng, H. C. (1967) Am. J. Physiol. 212, 284–290
11. LaRosa, J., Levy, R. I., Brown, W. V., and Fredrickson, D. S. (1973) Am. J. Physiol. 220, 785–791
12. Blanchette-Mackie, E. J., and Sow, R. O. (1971) J. Cell Biol. 51, 1–35
13. Scow, R. O., Hamosh, M., Blanchette-Mackie, E. J., and Evans, A. J. (1972) Lipids 7, 497–505
14. Komiyama, P., Bensadoun, A., and Wang Yang, M. W. (1976) J. Lipid Res. 17, 498–505
15. Assmann, G., Krauss, R. M., Fredrickson, D. S., and Levy, R. I. (1973) J. Biol. Chem. 248, 1999–1999
16. Iverius, P.-H. (1971) Biochem. J. 124, 677–688
17. Belfrage, P., and Vaughan, M. (1969) J. Lipid Res. 10, 341–344
18. Weber, K., and Ouborg, M. (1969) J. Biol. Chem. 244, 4406–4412
19. Zachariou, R. M., Zell, T. A., Morrison, J. H., and Woodlock, J. J. (1969) Anal. Biochem. 30, 148–152
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
21. Liu, T.-Y., and Chang, Y. H. (1971) J. Biol. Chem. 246, 2842–2848
22. Gray, W. R. (1972) Methods Enzymol. 25, 121–138
23. Summers, M. R., Smythers, G. W., and Orozslan, S. (1973) Methods Enzymol. 25, 621–628
24. Braun, V. and Schroeder, W. A. (1967) Arch. Biochem. Biophys. 116, 241–252
25. Freeze, H., and Loomis, W. F. (1977) J. Biol. Chem. 252, 820–824
26. Clamp, J. R., Bhatti, T., and Chambers, R. E. (1973) Methods Enzymol. 19, 229–243
27. Spiro, R. G. (1962) J. Biol. Chem. 237, 646–652
28. Warren, L. (1969) J. Biol. Chem. 244, 1971–1975
29. Liu, T.-Y., Gotechlich, E. C., Dunne, F. T., and Jonsen, E. K. (1971) J. Biol. Chem. 246, 4703–4712
30. Augustin, J., and Hassebach, W. (1973) Eur. J. Biochem. 35, 114–121
31. Hirs, C. H. W., Colowick, S. P., and Kaplan, N. O. (eds) (1967) Methods Enzymol. 21, 197–205
32. Edelman, G. M., Call, W. E., Waxdal, M. J., and Konisberg, W. H. (1968) Biochemistry 7, 1950–1958
33. Polch, J., Lees, M., and Blaone Stanley, G. H. (1957) J. Biol. Chem. 235, 497–509
34. Bartlett, G. R. (1969) J. Biol. Chem. 234, 466–468
35. Bensadoun, A., and Weinstein, D. (1976) Anal. Biochem. 70, 241–250
36. Brown, W. V., Levy, R. I., and Fredrickson, D. S. (1970) Biochim. Biophys. Acta 200, 573–595
37. Bensadoun, A., Ehnholm, C., Steinberg, D., and Brown, W. V. (1974) J. Biol. Chem. 249, 2220–2227
38. Segrest, J. F., and Jackson, R. L. (1971) Methods Enzymol. 18, 1–7
39. Capaldi, R. A., and Vanderkooy, G. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 930–932
40. Ehnholm, C., Kinnunen, P., and Huttunen, M. K. (1976) FEBS Lett. 52, 191–194
41. Fielding, P. E., Shore, V. G., and Fielding, C. J. (1974) Biochemistry 13, 4818–4823
42. Iverius, P.-H., and Ostlund-Lindqvist, A.-M. (1976) J. Biol. Chem. 251, 7791–7795
43. Egevad, T., and Oliveira, T. (1972) J. Biol. Chem. 247, 6212–6217
44. Twu, J. S., Garfinkel, A. S., and Schotz, M. (1975) Atherosclerosis 22, 463–473
45. Ehnholm, C., Kinnunen, P. K., Huttunen, J. K., Niskila, E. A., and Ohta, M. (1975) Biochem. J. 149, 649–655
46. Fielding, C. J. (1970) Biochim. Biophys. Acta 206, 109–117
47. Fielding, P. E., Shore, V. G., and Fielding, C. J. (1977) Biochemistry 16, 1896–1900
48. Chung, J., and Scana, A. M. (1977) J. Biol. Chem. 252, 4202–4203
49. Jansen, H., and Hulsmann (1975) Biochim. Biophys. Acta 398, 337–346
50. Baginsky, M. L., and Brown, W. V. (1977) J. Lipid Res. 18, 432–435
51. Brown, W. V., Levy, R. I., and Fredrickson, D. S. (1969) J. Biol. Chem. 244, 5687–5694
52. Plummer, T. H., Jr., and Sarda, L. (1973) J. Biol. Chem. 248, 7865–7869
53. Verger, R., DeHaas, G. H., Sarda, L., and Desnuelle, P. (1969) Biochim. Biophys. Acta 188, 272–282
54. Dziembor, E., Gryszkiewicz, J., and Ostrowski, W. (1970) Atherosclerosis 18, 272–282
55. Tashiro, Y., and Trevithick, J. R. (1977) Can. J. Biochem. 55, 249–256
56. Wetmore, S., Mahley, R. W., Brown, W. V., and Schachter, H. (1974) Can. J. Biochem. 52, 655–664
57. Elzinga, M., Maron, B. J., and Adelestein, R. S. (1976) Science 191, 94–95
A comparison of molecular properties of hepatic triglyceride lipase and lipoprotein lipase from human post-heparin plasma.

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J. Biol. Chem. 1978, 253:2912-2920.

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