

Enzyme-linked immunosorbent assay for rat hepatic triglyceride lipase

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Abstract A noncompetitive enzyme-linked immunosorbent assay to measure rat hepatic triglyceride lipase (H-TGL) was developed. Antibodies to rat H-TGL were purified from goat antisera by immunoadsorption on an H-TGL-Sepharose 4B column. Routinely, Immulon 2 Removawell strips were coated with the purified antibody overnight at 4°C. After blocking the wells with bovine serum albumin (BSA) for 2 hr at room temperature, standards (0.85 ng/ml–13.1 ng/ml) or samples were added to the wells and were incubated with the bound anti-rat H-TGL overnight at 4°C. The standards and samples had been pretreated with 5–20 mM SDS for 30 min at room temperature and were then diluted so that the final SDS concentration in the assay was 1 mM or less. The pretreatment with SDS was necessary to achieve maximal immunoreactivity. The sample incubation was followed by an overnight incubation at 4°C with an anti-rat H-TGL-horseradish peroxidase conjugate. Rat H-TGL was detected by the color development after the addition of 0.4 mg/ml of *o*-phenylenediamine in 0.01% H₂O₂, 0.1 M citrate phosphate, pH 5.0. A linear relationship was obtained between absorbance at 490 nm and the amount of highly purified rat H-TGL used as a standard. Inclusion of 1 M NaCl in the assay buffer (1% BSA, 0.05% Tween 20, 10 mM phosphate, pH 7.4) during the sample and conjugate incubations minimized non-specific interactions. Recoveries of purified rat H-TGL added to a rat liver perfusate sample ranged from 98.6% to 103%. The assay is specific for rat H-TGL. At a concentration 40-fold higher than the upper range of the assay, rat heart lipoprotein lipase exhibits only 0.2% cross-reactivity. Human hepatic triglyceride lipase, human lipoprotein lipase, and avian lipoprotein lipase were not detected in the assay. Using purified rat H-TGL as the standard, rat H-TGL was measured in dilutions of rat pre- and post-heparin plasma as well as a rat liver perfusate sample. This assay makes it possible to study the effects of hormones and metabolic intermediates on rat hepatic triglyceride lipase specific activity in cell culture systems. — Cisar, L. A., and A. Bensadoun. Enzyme-linked immunosorbent assay for rat hepatic triglyceride lipase. *J. Lipid Res.* 1985. 26: 380–386.

Supplementary key words ELISA • antibody

At present the function of hepatic triglyceride lipase (H-TGL) *in vivo* is unclear (1). H-TGL refers to the hepatic lipase which is characterized by an alkaline pH optimum and a resistance to high NaCl molarities. Suggested functions include the removal of cholesterol and phospholipids from HDL and LDL as they pass through

the liver (2). A recent report in the cynomolgus monkey proposes that H-TGL functions in concert with lipoprotein lipase (LPL) to convert very low density and intermediate density lipoproteins to low density lipoproteins (3).

In order to understand how H-TGL behaves at the molecular level in response to various agents such as hormones, it is necessary to be able to measure enzyme protein accurately as well as catalytic activity. It is for this purpose that the following enzyme-linked immunosorbent assay (ELISA) was developed.

MATERIALS AND METHODS

Purification of rat H-TGL

For the initial antibody production, rat H-TGL was purified as described by Jensen and Bensadoun (4). Rat livers were perfused with Krebs-Ringer bicarbonate buffer, pH 7.4, containing 10 units/ml heparin (porcine, Sigma, St. Louis, MO). The enzyme was purified by successive steps of heparin-Sepharose chromatography, DEAE-Sepharcel (Pharmacia, Uppsala, Sweden) anion exchange chromatography, and Ultrogel AcA34 (LKB, Bromma, Sweden) gel filtration chromatography. For the last immunological boosters, for preparation of an H-TGL-Sepharose 4B affinity column, and for development of the assay, rat H-TGL was purified by a modification of the above procedure. Trasylol (FBA Pharmaceuticals, New York, NY), at a final concentration of 300 units/ml, was added immediately after collection of the perfusate. Perfusates were stored at –70°C for up to 6 months before they were used in the purification. Thirty to ninety perfusates were loaded onto heparin-Sepharose 4B columns during one purification. Peak activity was concentrated

Abbreviations: H-TGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; HRP, horseradish peroxidase; PBS, 0.15 M NaCl, 10 mM phosphate, pH 7.4; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PAGE, polyacrylamide gel electrophoresis.

by $(\text{NH}_4)_2\text{SO}_4$ precipitation and loaded onto a DEAE-Trisacryl M (LKB) anion exchange column. Rat H-TGL was eluted with 0.2% Triton N-101, 0.05 M Tris, pH 7.2, into tubes containing a volume of 3 M NaCl to adjust the salt concentration in the fractions to 0.15 M NaCl. The final gel filtration column was omitted since its inclusion did not enhance purity. The yields were 340 μg , 698 μg , and 600 μg of rat H-TGL starting from the liver perfusates of 27 rats, 84 rats, and 88 rats, respectively. The purified enzyme had a specific activity of $11,200 \pm 2,200 \mu\text{eq}$ fatty acid per hr per mg protein (mean \pm SD from three purifications) and exhibited a major band at $57,400 \pm 200$ daltons and a faint band at $52,300 \pm 100$ daltons (mean \pm SD from four gels) in SDS-polyacrylamide gels (5) stained using either the Fairbanks procedure (6) or a commercial silver stain (Bio-Rad, Richmond, CA) (Fig. 1). The addition of Trasylol to liver perfusates appeared to reduce the concentration of the minor component in the final preparation. The specific activity of rat H-TGL observed in this study is lower than that reported previously (4). Several factors could explain this discrepancy. First, the purification was scaled up and more reliable protein

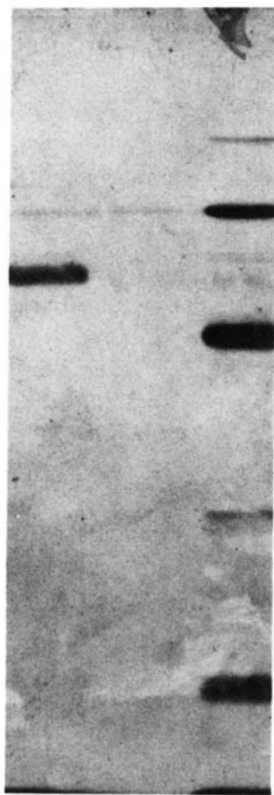


Fig. 1. SDS-polyacrylamide gel electrophoresis of purified rat H-TGL. The troughs from left to right contain: 1) 1.74 μg of DEAE-Trisacryl M-purified rat H-TGL; 2) sample buffer; and 3) 0.8 μg of phosphorylase b, 1.04 μg of bovine serum albumin, 1.84 μg of ovalbumin, 1.04 μg of carbonic anhydrase, 1 μg of soybean trypsin inhibitor, and 1.5 μg of lactalbumin. Protein was stained with a commercial silver stain (Bio-Rad).

determinations could be carried out on larger protein samples. It was also observed that when using the modified Lowry procedure of Bensadoun and Weinstein (7) with H-TGL, higher protein values were obtained if the trichloroacetic acid-precipitated protein was dissolved by incubation at 37°C in 0.1 M NaOH for 30 min. Secondly, elution from the DEAE-Trisacryl M was conducted with 0.2% Triton N-101 in the absence of NaCl. Although this modification improves purity as judged by SDS-PAGE and silver staining, it leads to a broader elution peak and exposure of dilute enzyme to a low ionicity buffer. Finally, even though Triton N-101 is a potent stabilizer of H-TGL (4), the concomitant presence of NaCl at a molarity of at least 0.15 M is necessary for optimal stabilization of the enzyme.

Antiserum production and anti-rat H-TGL immunoglobulin preparation

Antiserum to purified rat H-TGL was raised in a goat. Following an initial injection of 150 μg , two boosters of 164 μg and 130 μg were administered at 2-week intervals. Additional boosters were given periodically afterwards. To stimulate antibody production, the lipase was mixed with an equal volume of complete Freund's adjuvant (Difco, Detroit, MI) for each injection. The titer of the antiserum was determined by the method of Kompiang, Bensadoun, and Yang (8). A single antiserum pool with a titer of 3,600 μeq of fatty acid blocked per hr per ml was utilized in the development of this assay.

Immunodiffusion of the antiserum with the liver perfusate starting material and the highly purified enzyme was performed on IFD Cell II dishes (Cordis, Miami, FL) according to the method of Ouchterlony (9). To concentrate the antigenic material, the samples were dialyzed against 0.001% (w/v) NaN_3 and lyophilized. The lyophilized material from 50 ml of liver perfusate which had exhibited an activity of 11 μeq of fatty acid per hr per ml was resuspended in 1.2 ml of 0.1% (w/v) Triton N-101, 0.05 M Tris, pH 7.2. Purified rat H-TGL (12 μg) was resuspended in 160 μl of water. The antiserum was diluted with 0.15 M NaCl, 10 mM phosphate, (PBS), pH 7.4. Immunodiffusion of the antiserum yielded a single precipitin line against both the crude H-TGL in rat liver perfusate and the highly purified rat H-TGL.

Specific anti-rat H-TGL immunoglobulins were purified on an H-TGL-Sepharose 4B column. Purified rat H-TGL (332 μg) was coupled to 12 ml of CNBr-activated Sepharose 4B (Pharmacia) (10). For efficient coupling, the detergent was removed from the purified enzyme with an affi-gel heparin (Bio-Rad) column. DEAE-Trisacryl M purified rat H-TGL in 0.15 M NaCl, 0.2% Triton N-101, 0.05 M Tris, pH 7.2, was diluted with an equal volume of 30% glycerol, 10 mM phosphate, pH 7.0, and loaded onto an affi-gel heparin column (0.9 cm \times 5 cm) that had been

equilibrated with the same buffer. The column was washed extensively with the equilibration buffer and with 0.3 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.0. The enzyme was eluted with 0.75 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.0. Purified rat H-TGL (332 $\mu\text{g}/22\text{ ml}$) was dialyzed against 10% glycerol, 0.2 M NaHCO_3 , pH 7.0, immediately before it was coupled to the Sepharose 4B.

The antiserum, the immunoabsorbent column, and the purified immunoglobulins were handled as described by Cheung, Bensadoun, and Cheng (11). When 2 ml of the antiserum pool exhibiting a titer of 3,600 μeq of fatty acid blocked per hr per ml was loaded onto the rat H-TGL-Sepharose 4B column, 0.69 mg of purified immunoglobulins was recovered. The purified immunoglobulins were still able to recognize rat H-TGL, as was demonstrated by their continued ability to inhibit enzyme activity. The protein from several runs was combined and concentrated to 1.65 mg/ml for storage. SDS-polyacrylamide gels of the purified immunoglobulins showed two major bands corresponding to the heavy and light chains of IgG. Over time the capacity of the immunoabsorbent column decreased. Recently, it was discovered that the capacity of the column could be restored by the inclusion of either 4 mM CHAPS or 0.1% Triton N-101 in the 0.5 M NaCl, 0.2 M Tris, pH 8.0, equilibration buffer. The detergents were removed from the column by extensive washing prior to antibody purification.

Preparation of the conjugate

The purified anti-rat H-TGL immunoglobulins from goat were conjugated to horseradish peroxidase (HRP) using the two-step glutaraldehyde method of Avrameas and Ternynck (12). Two ml of purified antibodies (2.2 mg/ml) in PBS, pH 7.4, was dialyzed against 0.15 M NaCl and added to glutaraldehyde-activated HRP. After blocking the unreacted groups with lysine and removing free HRP by $(\text{NH}_4)_2\text{SO}_4$ precipitation, the HRP-immunoglobulin conjugate was filtered (0.22 μm filter, Millipore, Bedford, MA) and stored in 1% (w/v) bovine serum albumin (BSA), PBS, pH 7.4, at -20°C .

Assay conditions for a noncompetitive ELISA to measure rat H-TGL

Immulon 2 Removawell strips (Dynatech, Alexandria, VA) were used in the development of the assay. Wells were coated overnight at 4°C with 200 μl of antibody that had been diluted to 2.75 $\mu\text{g}/\text{ml}$ with 0.02% NaN_3 , 0.1 M carbonate-bicarbonate, pH 9.6. The solution was removed by aspiration and the wells were washed three times with 0.05% (w/v) Tween 20, PBS, pH 7.4. This removal and washing procedure followed all subsequent incubations except the final incubation with substrate. To decrease

nonspecific binding, the wells were blocked with 300 μl of 1% BSA, 0.05% Tween 20, PBS, pH 7.4, for 2 hr at room temperature. Blocking was followed by an overnight incubation at 4°C of the bound anti-rat H-TGL with 200 μl of highly purified rat H-TGL standards or samples. Prior to their addition to the wells, a rat H-TGL stock solution and biological samples were preincubated in 20 mM sodium dodecyl sulfate (SDS) at room temperature for at least 30 min. Standards and samples were further diluted with 1 M NaCl, 1% BSA, 0.05% Tween 20, 10 mM phosphate, pH 7.4, such that the final concentration of SDS in the assay was 0.4 mM. Rat H-TGL standards (0.85 ng/ml–13.1 ng/ml) were freshly prepared for each assay from a stock solution of purified rat H-TGL ($34.9 \pm 0.4\ \mu\text{g}/\text{ml}$) in 0.15 M NaCl, 0.2% Triton N-101, 0.05 M Tris, pH 7.2. The rat H-TGL stock was stored in 50 μl aliquots at -70°C . Following the sample incubation, the wells were incubated with 200 μl of diluted conjugate overnight at 4°C . The conjugate dilution buffer was the same as the sample dilution buffer, 1 M NaCl, 1% BSA, 0.05% Tween 20, 10 mM phosphate, pH 7.4. The final incubation with 200 μl of substrate solution, which included 0.4 mg/ml *o*-phenylenediamine in 0.01% H_2O_2 , 0.1 M citrate phosphate buffer, pH 5.0 (13), lasted exactly 30 min. The reaction was stopped by the addition of 50 μl of 2.5 M H_2SO_4 to each well. The plates were read at 490 nm on a Microelisa Auto Reader (Dynatech). The dilution of the conjugate in the assay was chosen by its ability to optimize the color density obtained with 13.1 ng/ml rat H-TGL within the detection range of the spectrophotometer. A 1:4000 dilution of conjugate was used in this study. Aliquots of the sample dilution buffer (200 μl) were added as samples to the first and last wells of each row as controls for nonspecific binding of the conjugate. The absorbance of these control wells was one-sixth the absorbance of the wells incubated with 13.1 ng/ml rat H-TGL. Nonspecific binding was subtracted from the total absorbance of a given sample to yield specific absorbance.

The accuracy of the assay depended on a careful washing procedure. After each incubation the solution was completely removed by aspiration with an eight-channel manifold. The wells were quickly washed three times with 0.05% Tween 20, PBS, pH 7.4, using a Nunc-Immuno Wash 12 (Nunc, Denmark). The wells were never left entirely dry. Prior to the addition of either the blocking buffer or the diluted conjugate, the final wash was completely removed one row at a time using the manifold. Immediately afterwards the appropriate solution was delivered to that row using a Titer Tek (Flow, McLean, VA) twelve-channel pipette. Before the addition of samples or standards, the final wash was removed from each well individually with a pipette attached to a water aspirator. The appropriate sample was then added immediately to that well.

Samples assayed

Rat liver perfusate was obtained as described previously (4). Plasma samples were obtained from 360–420 g male Sprague-Dawley rats. The rats were anesthetized with ether and blood was drawn from the subclavian vein with a heparinized syringe. One hundred microliters of heparin (1,000 units/ml, porcine, Sigma) in 0.15 M NaCl was injected into the vein and 2 min later blood was drawn from the abdominal aorta. Plasma was isolated by spinning the blood at 1,000 *g* for 10 min at 4°C. Plasma samples were stored at –20°C until they were assayed.

Human H-TGL and human LPL were partially purified by heparin-Sepharose chromatography from post-heparin plasma. Enzyme protein in the samples was estimated based on a final specific activity of 20,000 μ eq of fatty acid per hr per mg protein for the purified lipases (Cheng and Bensadoun, unpublished data). Chicken adipose LPL was purified to homogeneity as described by Cheung et al. (11). The preparation used in this study had a final specific activity of 9,900 μ eq of fatty acid per hr per mg protein. Rat LPL was partially purified from rat heart tissue (14, 15). Heart tissue acetone powders were prepared as previously described for adipose tissue (16). Acetone powders (50 mg/ml buffer) were extracted in 0.5 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.4, containing 1 unit heparin/ml. Extracts were spun at 20,000 *g* for 10 min, diluted with 10% glycerol, 10 mM phosphate, pH 7.0, and respun. The supernatant was applied to an affi-gel heparin (Bio-Rad) column that had been equilibrated with 0.3 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.0. The column was washed extensively with the equilibration buffer and with 0.75 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.0, before a step elution with 1.5 M NaCl, 30% glycerol, 10 mM phosphate, pH 7.0. Rat heart LPL protein was estimated using a specific activity of 10,000 μ eq of fatty acid per hr per mg protein (13). In order to test whether high levels of human H-TGL (644 ng/ml), human LPL (683 ng/ml), chicken adipose LPL (500 ng/ml), and rat heart LPL (538 ng/ml) would be detected in the ELISA, samples and standards were pre-incubated in 5 mM SDS and were diluted to a final concentration of 1 mM SDS in the assay.

Other procedures

Control immunoglobulins were partially purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation from pre-immunization goat serum (17). Immunoglobulins were stored in PBS, pH 7.4, at –70°C. Lipase activity was assayed with a gum arabic-stabilized triolein emulsion (18). H-TGL activity was measured in 1 M NaCl while LPL activity was measured in 0.1 M NaCl in the presence of apoC-II-containing serum. Protein was determined by a modified Lowry procedure using crystallized bovine serum albumin as standard (7). To ensure that the protein pellets were fully

dissolved, 100 μ l of 0.1 N NaOH was added to rat H-TGL samples and standards. These were then incubated at 37°C for 30 min prior to the addition of the Lowry reagents.

RESULTS AND DISCUSSION

Standard curve for the noncompetitive ELISA for rat H-TGL

The protocol described in detail in Materials and Methods was routinely followed. For standards, highly purified rat H-TGL was diluted to final concentrations that ranged from 0.85 ng/ml to 13.1 ng/ml. A typical standard curve is shown in Fig. 2. Absorbance at 490 nm increased linearly as a function of the rat H-TGL added. When the wells were coated with control immunoglobulins rather than the antibodies against rat H-TGL, there was no detectable absorbance for rat H-TGL within the range of the standard curve.

Characteristics of the assay

The washing procedure was critical to the accuracy of the assay. As explained in the description of the method, care was taken to minimize and standardize the length of time the wells of the microtiter plates remained empty. Partial drying and denaturation of the immunoglobulins could account for the observed decreased reactivity when wells are empty for extended periods of time.

Incubation conditions were chosen to optimize the assay's sensitivity and to conserve reagents. Sodium chloride (1 M) was included in the sample and in conjugate

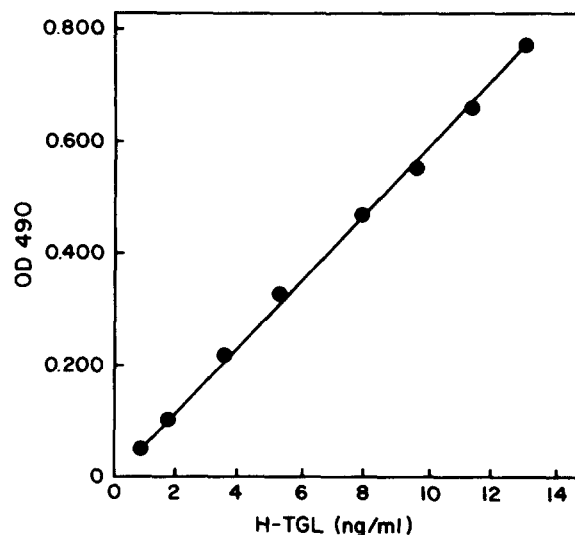


Fig. 2. Standard curve. Highly purified rat H-TGL (0.85 ng/ml–13.1 ng/ml) was assayed as described in Materials and Methods. Each point represents the average of duplicates. At 5.25 ng/ml of H-TGL, the duplicate values differed by 4%.

incubations in order to decrease nonspecific interactions. In studies on the effect of denaturation of H-TGL on detection by the assay, it was noted that preincubation of both standards and biological samples with 5 to 20 mM sodium dodecyl sulfate yielded higher absorbances than control samples (Fig. 3 and Fig. 4). A 30-min incubation with 5 to 20 mM SDS at 30°C (19) was sufficient to inactivate rat H-TGL liver perfusates and to maximize the increase in absorbance (Fig. 4), while final SDS concentrations of 1 mM or less did not interfere in the assay. Similar data were obtained with room temperature incubations. Essentially, SDS pretreatment made both active and inactive rat H-TGL equally recognizable to the antibodies. Thus, samples and standards were pretreated with 20 mM SDS in an attempt to eliminate detection differences due to varying conformational states of rat H-TGL. A second advantage of the SDS pretreatment was the decreased variability in the standard curve (Fig. 3). The sample standard deviations were 0.005 and 0.014 OD₄₉₀ units for the standard curves with and without SDS, respectively.

Sensitivity and specificity of the assay

The assay was reproducible within the range of the standard curve. Variability in measuring rat H-TGL in samples run on the same day was 9.8% for a sample containing 1.93 ng/ml rat H-TGL and 3.4% for a sample containing 13.4 ng/ml rat H-TGL. Measurements of two samples against four different standard curves varied

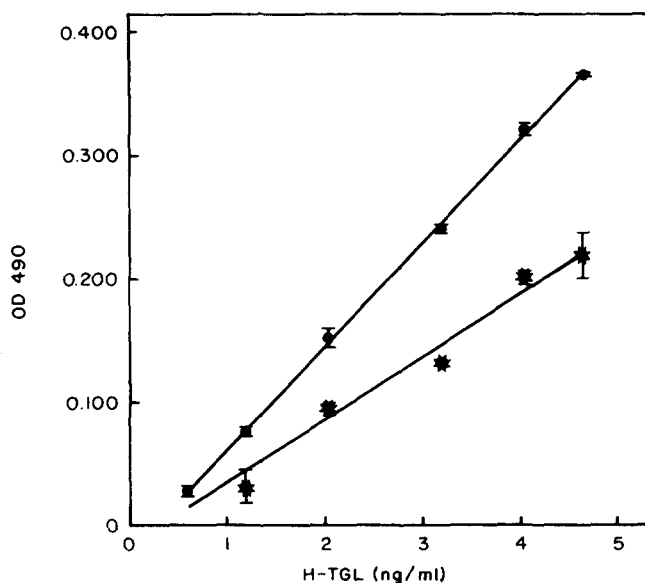


Fig. 3. The effect of SDS preincubation on the standard curve. A rat H-TGL stock solution was preincubated for 30 min at room temperature either with (●) or without (*) 20 mM SDS. The highly purified enzyme was further diluted (0.60 ng/ml–4.65 ng/ml) and assayed as described in Materials and Methods. Each point represents the mean \pm standard deviation of three replicates.

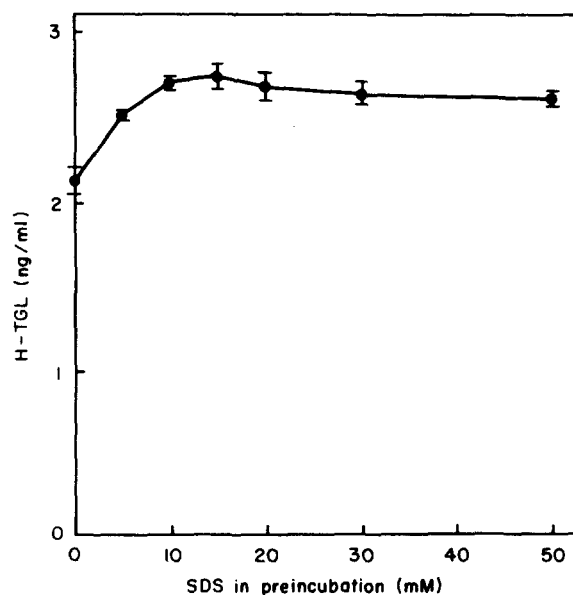


Fig. 4. The effect of SDS preincubation on the measurement of rat H-TGL in liver perfusate. Rat H-TGL in liver perfusate (0.36 μ g/ml) was diluted 3:4 such that the SDS concentration in the preincubation mixture ranged from 5 mM to 50 mM. The samples were preincubated at 30°C for 30 min. Samples were diluted 1:100 in the assay so that final SDS concentrations ranged from 0.05 mM to 0.5 mM for the 5 mM to 50 mM preincubations, respectively. Samples and standards were assayed as described in the text. Samples were compared to standards that had been preincubated with 20 mM SDS and that were diluted to 0.2 mM SDS in the assay. Each point represents the mean \pm standard deviation of three replicates.

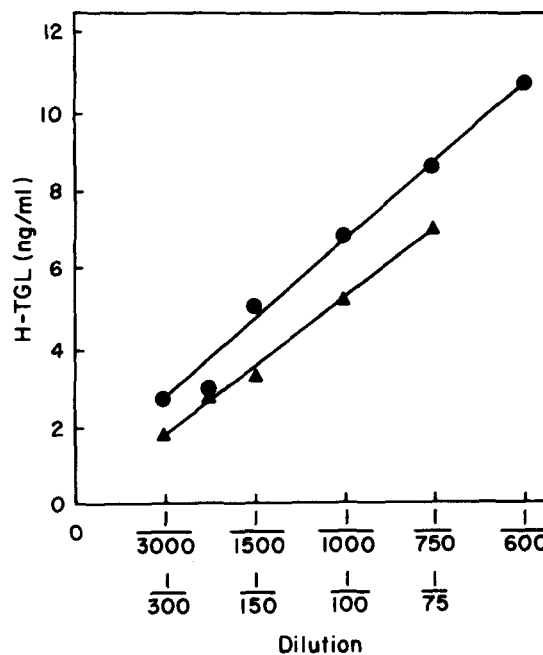


Fig. 5. Dilution of crude samples. A liver perfusate sample (\blacktriangle - \blacktriangle) and a post-heparin plasma sample (\bullet - \bullet) containing 0.53 μ g/ml and 6.85 μ g/ml rat H-TGL, respectively, were diluted such that the rat H-TGL measured would fall within the range of the standard curve. Samples were assayed as described in the text. Each point represents the average of duplicates. At 1:100 and 1:1000 dilutions for the liver perfusate and post-heparin plasma samples, respectively, the duplicate values differed by less than 5%.

TABLE 1. Specificity of the ELISA for rat hepatic triglyceride lipase

Sample	Enzyme Added	Rat H-TGL Determined	Recovery
	ng/ml	ng/ml	%
Human H-TGL	644 ^a	0	0
Human LPL	683 ^a	0	0
Chicken adipose LPL	500 ^b	0	0
Rat heart LPL	538 ^c	0.91 ^d	0.2

^aEnzyme protein was estimated based on a specific activity of 20,000 μeq of fatty acid per hr per mg protein for the purified human lipases.

^bThe chicken adipose LPL used in this study had a specific activity of 9,900 μeq of fatty acid per hr per mg protein.

^cA specific activity of 10,000 μeq of fatty acid per hr per mg protein was assumed in calculating the rat heart LPL protein concentration.

^dThe amount of rat H-TGL protein detected represents the average of duplicates.

15.1% for 2.31 ng/ml rat H-TGL and 9.1% for 12.8 ng/ml rat H-TGL.

To further test the reproducibility of measurements made with this assay, a liver perfusate sample and a post-heparin plasma sample were diluted to different extents and the amount of rat H-TGL present was determined by comparison to a standard curve (Fig. 5). The samples contained $0.53 \pm 0.03 \mu\text{g/ml}$ and $6.85 \pm 0.79 \mu\text{g/ml}$ of rat H-TGL, respectively. When varying amounts of purified rat H-TGL (0.26 ng to 1.16 ng) were added to 0.66 ng of liver perfusate rat H-TGL in a final volume of 200 μl , recoveries ranged from 98.6% to 103%.

In order to examine the specificity of the assay, samples containing other lipases were assayed (Table 1). The partially purified lipases were not concentrated enough to be preincubated with 20 mM SDS and subsequently diluted to a concentration of 1 mM SDS or less in the assay. For these measurements, standards and samples were preincubated with 5 mM SDS. Human H-TGL, human lipoprotein lipase, and chicken adipose lipoprotein lipase

were not detected at all in the assay, while rat heart lipoprotein lipase in 40-fold excess of the upper range of the assay exhibited 0.2% cross-reactivity. Within the 0.85 ng/ml to 13.1 ng/ml range of the assay, there was no detectable absorbance for rat heart lipoprotein lipase. Thus, this ELISA appears to be highly specific for rat H-TGL.

A liver perfusate sample and pre- and post-heparin plasma samples were assayed for rat H-TGL enzyme protein (Table 2). Some interesting differences in specific activity were observed. The specific activity of the enzyme in pre-heparin plasma was $1,300 \pm 100 \mu\text{eq}$ of fatty acid per hr per mg protein versus 22,000 μeq of fatty acid per hr per mg protein for liver perfusate. Transport of H-TGL and differences in stability in various sites could explain the observed differences in specific activity. Binding of lipoprotein lipase to endothelial cells in culture at 37°C for 3 hr stabilizes enzyme catalytic activity (20) whereas the soluble enzyme is rapidly inactivated at the same temperature. Similarly, binding of H-TGL to the liver sinusoidal endothelium might stabilize its activity. This could account for the high specific activity of the liver perfusate enzyme. Pre-heparin plasma contains secreted enzyme which has circulated for variable length of time and is in part inactivated. The specific activity of the enzyme purified from liver perfusate ($11,200 \pm 2,200 \mu\text{eq}$ of fatty acid per hr per mg protein) is about half that measured in the crude perfusate. This suggests that some inactivation occurs in the process of purification before the DEAE-Trisacryl M step where the enzyme is eluted in the presence of the stabilizing non-ionic detergent, Triton N-101.

Since Trasylol and heparin were present in the sample measured, it was validated that neither Trasylol at a level of 10 units/ml nor heparin up to a level of 50 units/ml interfered in the assay.

TABLE 2. Rat hepatic triglyceride lipase activity and protein measurements

Sample	Activity ^a	Enzyme Protein ^b	Specific Activity
	μeq fatty acid/hr/ml	$\mu\text{g/ml}$	μeq fatty acid/hr/mg protein
Rat ₁			
Post-heparin plasma	63.1 ± 1.8^c	6.85 ± 0.79^c	9,200
Pre-heparin plasma	0.16^d	0.13^d	1,200
Rat ₂			
Post-heparin plasma	31.6 ± 1.5^c	3.47 ± 0.24^c	9,100
Pre-heparin plasma	0.14^d	0.11^d	1,300
Rat ₃			
Post-heparin plasma	66.3 ± 3.6^c	9.36^d	7,100
Pre-heparin plasma	0.13^d	0.09^d	1,400
Liver perfusate	13.04^d	0.58 ± 0.03^c	22,000

^aActivity was assayed with a gum arabic stabilized triolein emulsion.

^bEnzyme protein was determined by the ELISA.

^cMeasurements represent the mean \pm the standard deviation in 4 to 14 replicates.

^dMeasurements represent the average of duplicates.

The noncompetitive ELISA procedure described in this paper should be sufficiently sensitive to study the regulation of hepatic triglyceride lipase in whole animal studies and cell culture experiments. ■■

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REFERENCES

1. Nilsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49**: 667-693.
2. Kuusi, T., P. K. J. Kinnunen, and E. A. Nikkila. 1979. Hepatic endothelial lipase antiserum influences rat plasma low and high density lipoproteins in vivo. *FEBS Lett.* **104**: 384-388.
3. Goldberg, I. J., N. A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70**: 1184-1192.
4. Jensen, G. L., and A. Bensadoun. 1981. Purification, stabilization, and characterization of rat hepatic triglyceride lipase. *Anal. Biochem.* **113**: 246-252.
5. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227**: 680-685.
6. Fairbanks, G., T. L. Steck, and D. F. H. Wallach. 1971. Electrophoretic analysis of major polypeptides of human erythrocyte-membrane. *Biochemistry.* **10**: 2606-2619.
7. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70**: 241-250.
8. Kompiang, I. P., A. Bensadoun, and M. W. W. Yang. 1976. Effect of an anti-lipoprotein lipase serum on plasma triglyceride removal. *J. Lipid Res.* **17**: 498-505.
9. Muñoz, J. 1971. Precipitation analysis by diffusion in gels: quantitative analysis of antigen-antibody reactions in gels. *Methods Immunol. Immunochem.* **3**: 146-160.
10. March, S. C., I. Parikh, and P. Cuatrecasas. 1974. A simplified method for cyanogen bromide activation of agarose by affinity chromatography. *Anal. Biochem.* **60**: 149-152.
11. Cheung, A. H., A. Bensadoun, and C-F. Cheng. 1979. Direct solid phase radioimmunoassay for chicken lipoprotein lipase. *Anal. Biochem.* **94**: 346-357.
12. Avrameas, S., and T. Ternynck. 1971. Peroxidase labeled antibody and Fab conjugates with enhanced intracellular penetration. *Immunochemistry.* **8**: 1175-1179.
13. Wolters, G., L. J. Kuijpers, J. Kačaki, and A. Schurs. 1976. Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. *J. Clin. Pathol.* **29**: 873-879.
14. Chung, J., and A. M. Scanu. 1977. Isolation, molecular properties, and kinetic characterization of lipoprotein lipase from rat heart. *J. Biol. Chem.* **252**: 4202-4209.
15. Benson, J. D., and A. Bensadoun. 1977. Response of adipose tissue lipoprotein lipase to fasting in the chicken and the rat—a species difference. *J. Nutr.* **107**: 990-997.
16. Korn, E. D., and T. W. Quigley, Jr. 1957. Lipoprotein lipase of chicken adipose tissue. *J. Biol. Chem.* **226**: 833-839.
17. Livingston, D. M. 1974. Immunoaffinity chromatography of proteins. *Methods Enzymol.* **34**: 723-731.
18. Bensadoun, A., and T. L. Koh. 1977. Identification of an adipose tissue-like lipoprotein lipase in perfusates of chicken liver. *J. Lipid Res.* **18**: 768-773.
19. Baginsky, M. L., and W. V. Brown. 1979. A new method for the measurement of lipoprotein lipase in postheparin plasma using sodium dodecyl sulfate for the inactivation of hepatic triglyceride lipase. *J. Lipid Res.* **20**: 548-556.
20. Cheng, C. F., G. M. Oosta, A. Bensadoun, and R. D. Rosenberg. 1981. Binding of lipoprotein lipase to endothelial cells in culture. *J. Biol. Chem.* **256**: 12893-12898.