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Lipoprotein Lipase: Properties of the Enzyme Isolated from Post-Heparin Plasma†

P. E. Fielding, V. G. Shore, and C. J. Fielding*

ABSTRACT: Lipoprotein lipase purified from rat post-heparin plasma was characterized in terms of its amino acid, carbohydrate, and lipid composition. Molecular weight determinations by several procedures indicate a monomeric molecular weight of about 37,000; apparent dimer and tetramer forms were also identified. The purified lipase is a glycoprotein, but it does not contain heparin. It did not bind heparin in solution or covalently attached to agarose beads. The purified lipase retained approximately 1 mol of phospholipid/mol of protein.

Heparin releases from the endothelial membrane of the blood capillaries of the extrahepatic tissues a lipase (post-heparin lipoprotein lipase) active on the triglycerides of the natural lipoprotein substrates (chylomicrons, very low density lipoproteins). This enzyme has been purified in a number of laboratories (Fielding, 1969, 1970; Nilsson-Ehle *et al.*, 1971; Yasuoka and Fujii, 1971). The protein moiety of the lipoprotein substrates stimulates the hydrolysis of synthetic neutral lipid substrates by the purified enzyme (Havel *et al.*, 1973). This lipase has a high specificity for reaction with emulsified substrates (Fielding, 1973). It is released from its membrane site by high molecular weight polyanions, in particular polysaccharide sulfates such as heparin (Korn, 1957). In an attempt to define further the molecular basis of these properties we have undertaken a chemical and physical characterization of this enzyme, which is the subject of the present report.

Experimental Section

Materials

Unlabeled heparin was purchased from Invenex Company, San Francisco. [*N-sulfonate*-^{35}S]Heparin (initial radioactivity 13.5 mCi/g) (mol wt 14,500 by sedimentation equilibrium for $\bar{v}$ = 0.47 cm^3/g) (Barlow *et al.*, 1961) was from Amersham-Searle, Chicago, and was used without dilution with unlabeled heparin. No difference was found in the amount or properties of lipase released by unlabeled or radioactive heparin species. Linolenic acid (Sigma Chemical Co., St. Louis, Mo.), 99% pure by thin-layer chromatography, was used as the potassium salt after conversion with aqueous KOH. Sodium deoxycholate was from Mann Research, New York, N. Y., and Intralipid 20% triglyceride emulsion with lecithin was the gift of Vitrum AB, Stockholm, Sweden. Animal plasma donors were male Sprague-Dawley rats, fed ad libitum; 10–12 ml of plasma was obtained from each animal of 400–500 g body weight by aortic cannulation.

Methods

Lipase Preparation. Lipoprotein lipase was purified from blood plasma of rats obtained 3–5 min after the injection of heparin (1 mg/kg body weight). Lipase activity of plasma and at various stages of purification was assayed with triglyceride–lecithin emulsion in the presence of recalcified

† From the Cardiovascular Research Institute, University of California, San Francisco, California 94143, and the Biomedical Division, Lawrence Livermore Laboratory, University of California, Livermore, California 94550. *Received May 23, 1974.* This work was supported in part by the U. S. Public Health Service (HL 14237) and the American Heart Association (71-1064 and 73-708) and in part by the U. S. Atomic Energy Commission. P. E. F. was a Postdoctoral Trainee of the U. S. Public Health Service (HL 5251) and C. J. F. is Established Investigator (70-142) of the American Heart Association.

citrated rat plasma (Fielding, 1968). Purification was by a modification of the method previously described (Fielding, 1969). Lipase activity, typically from 100 ml of post-heparin plasma, was adsorbed to triglyceride emulsion (5 mg of triglyceride/ml of plasma) by incubation at 37° for 7 min (Anfinsen and Quigley, 1953), then layered under an equal volume of 0.05 M NH_4OH–NH_4Cl buffer (pH 8.3) containing 10% w/v sucrose. The preparation was then centrifuged for 45 min at 30,000 rpm in the 30-rotor of the Spinco L3-50 preparative ultracentrifuge at 2–4°. The floating fatty layer, containing about 60% of total post-heparin lipase activity, was recentrifuged five times under the same conditions, and the final supernatant material was diluted with an equal volume of 0.05 M ammonia buffer containing 0.5 mM potassium linolenate and 10 mM deoxycholate. The lipase was quantitatively released into solution, and the soluble fraction separated from floating lipid and insoluble precipitated protein by centrifugation at 2–4° in the SW-41 rotor at 25,000 rpm for 1 hr. The clear aqueous solution containing the enzyme was generally mixed with 20 mg/ml of Dextran T-500 (Pharmacia, Uppsala, Sweden) and the mixture was precipitated with 10 volumes of absolute acetone at 0°. In measurements of the carbohydrate content of lipoprotein lipase, the precipitation with dextran was omitted from the procedure. After two washings with the same solvent, the precipitate was washed with two further portions of acetone at room temperature, and then with two portions of diethyl ether. The precipitate was dried under vacuum. Acetone–ether extraction did not increase purification (about 1500-fold at this step) but removed phospholipid and unesterified cholesterol from the active fraction; recovery of lipase activity at this step was complete. The precipitate was then redissolved in 0.05 M ammonia buffer, 0.5 mM linolenate, and 10 mM deoxycholate (50 μg of protein/ml of buffer) and mixed with one-fifth volume of 0.2 M calcium phosphate gel. Following removal of the supernatant fluid after 30 min by centrifugation, the gel was washed successively with two portions of ammonia buffer containing linolenate, deoxycholate, and 0.1 M potassium oxalate, then with ammonia buffer–oxalate, then finally with one-fourth volume of 50 mM sodium citrate, 0.5 mM linolenate in ammonia buffer. (Omission of the fatty acid in the elution buffer permitted complete recovery of adsorbed protein but with only about one-fourth of the specific activity of the usual procedure.) Recovery of lipase through the gel procedure was about 50%.

Post-heparin hepatic lipase and acetone-extracted adipose tissue were prepared as previously described (Fielding, 1972; Salaman and Robinson, 1966).

Lipoprotein Isolation. Rat serum lipoproteins were prepared by ultracentrifugation in the presence of 0.1% w/v ethylenediaminetetraacetic acid (pH 7.0). Very low density lipoprotein was collected by ultracentrifugation without adjustment of serum density at 40,000 rpm for 24 hr at 2–4° in the 40.3 rotor. The floating lipoprotein fraction was recentrifuged three times from 0.154 M NaCl under the same conditions. Low density ($1.03 < d < 1.04$) and high density ($1.063 < d < 1.21$) lipoproteins were similarly isolated by repeated ultracentrifugation between the required density limits (Havel *et al.*, 1955).

Immunoassay. Immunological purity of lipoprotein lipase was assessed with rabbit antisera to the enzyme protein, purified rat serum lipoproteins, and rat albumin (Sigma, recrystallized). Anti-lipase antibody was prepared by injection of 100 μg of antigen protein in 3 ml of ammonia buffer mixed with an equal volume of Freund's complete adjuvant into the footpads. Injection was repeated intradermally after 10 days and the animals were bled 2 weeks later. Antibodies to rat serum lipoproteins and albumin (Sigma, recrystallized) were prepared similarly. Double immunodiffusion was carried out by the Ouchterlony technique in 1% agar plates containing 0.05 M barbital buffer (pH 8.6) and 0.01% merthiolate as preservative.

Polyacrylamide Gel Disc Electrophoresis. Disc gel electrophoresis was carried out at pH 8.9 (Davis, 1964). Polyacrylamide gels without sodium dodecyl sulfate were run at 4° and 2 mA/tube, then stained with 0.02% Coomassie Blue in 12.5% trichloroacetic acid; in some experiments the gels were sliced into 2-mm fractions which were then assayed for lipase with ^{14}C-labeled rat serum very low density lipoprotein (Felts and Masoro, 1959; Fielding and Higgins, 1974). Assay was for 2 hr at 25°. Electrophoresis in the presence of sodium dodecyl sulfate was by the method of Dunker and Reuckert (1969). In these experiments, lipase was pretreated by exposure for 3 hr to 5% detergent in ammonia buffer (pH 8.3) containing 0.1 M 2-mercaptoethanol at room temperature or at 37°. In some experiments the protein was previously carboxymethylated after reduction with iodoacetamide. Gels were fixed overnight with 20% 5-sulfosalicylic acid, stained with Coomassie Blue as described above, and destained with 10% trichloroacetic acid.

Molecular Weight Determinations. Disc electrophoresis in 6-cm gels was used also for estimation of molecular weight of lipase protein; its mobility, a function of molecular weight (Dunker and Reuckert, 1969), was compared with those of pure standard proteins: human serum albumin (Sigma, crystallized, 68,000); pepsin (Sigma, twice recrystallized, 35,500); trypsin (Sigma, twice recrystallized, 23,800); lysozyme (Sigma, three times recrystallized, 14,000).

Ultracentrifugal studies of purified lipase were carried out using the Beckman Model E analytical ultracentrifuge at 17,980 rpm and 9° with Rayleigh interference optics for sedimentation equilibrium studies. Runs were carried out at a series of protein concentrations in the range 0.1–0.6 mg/ml. Meniscus concentration was determined by fringe displacement (Charlwood, 1957). Liquid columns were 3 mm high, and a gradient-forming centerpiece was used to reduce the time required to reach equilibrium. Equilibrium data were analyzed from plots of $1/r\,dc/dr$ *vs.* Δc according to Van Holde and Baldwin (1958) where r is the radial distance, c the concentration in fringes, and Δc the concentration difference from the reference point at the meniscus. The derivative was obtained by least-squares analysis (Yphantis, 1964) and the Z-average molecular weight from the slope of the derivative. This technique of analysis was preferred to plotting log c *vs.* r^2 because it gives a more sensitive measure of association than does the weight-average molecular weight. Partial specific volume of the protein was determined from the chemical analysis of the purified protein. Enzyme to be analyzed by sedimentation equilibrium procedures was dialyzed overnight against 0.2 M NaCl–0.05 M ammonia buffer (pH 8.3) at 0–2°. About 80% of lipase activity was lost during this period, without visible precipitation.

Most of the remaining activity could be recovered from the utracentrifuge cell after the run.

Molecular weight was also determined by Sephadex gel chromatography using 100 × 2 cm columns of G-150 gel equilibrated with 0.05 M ammonia buffer containing 0.5

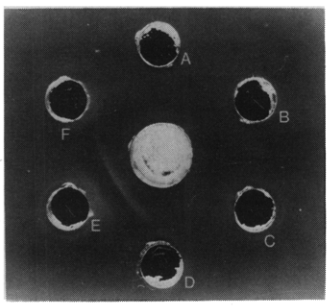

FIGURE 1: Immunodiffusion pattern of lipase protein (88 μg of protein) with antibodies against rat serum lipoprotein classes and albumin. Clockwise from the top: (A) anti-rat albumin; (B) anti-rat high density lipoprotein; (C) anti-rat low density lipoprotein; (D) anti-rat very low density lipoprotein; (E) anti-rat lipoprotein lipase; (F) control serum before immunization.

mM linolenate. The results obtained did not differ from those reported in a previous publication (Fielding, 1969) and are discussed in a later section.

Heparin–Lipoprotein Lipase Interaction. Linear sucrose-density gradients (5–20% sucrose in 0.05 M ammonia buffer–0.5 mM linolenate (pH 8.3)) contained a uniform initial concentration of [^{35}S]heparin in a volume of 4.9 ml in the tubes of an SW-39 rotor; 0.1 ml of lipase solution (0.2 mg/ml), preincubated with the same heparin concentration for 2 hr at 0°, was layered on the gradient, and centrifuged for 15 hr at 38,000 rpm at 2°. After completion of the run, 0.1-ml fractions were collected from the bottom of the gradients and each was assayed for lipase acitivity and for radioactivity. Liquid scintillation procedures for determination of radioactivity have been reported previously (Fielding and Fielding, 1971). For investigation of lipase binding to immobilized heparin, a covalent heparin–agarose complex was prepared with Sepharose 4B–cyanogen bromide complex (Pharmacia, New York, N. Y.) in the proportions 3.2 mg of heparin/ml of gel packed volume (Iverius, 1971). The complex was washed to remove unbound heparin, then incubated with 1 M ethanolamine-HCl (pH 8.0) to neutralize unreacted gel residues. The heparin-gel complex was then washed with 0.05 M ammonia buffer before use in affinity column chromatography.

Chemical Analyses. Lipid analyses were carried out with chloroform–methanol extracts of purified lipase prepared according to Folch *et al.* (1957). After separation of the major lipid classes by thin-layer chromatography (Fielding and Higgins, 1974) determination of individual classes was carried out by laboratory micromodifications of the methods of Carlson (1963) for neutral glycerides, Bartlett (1959) for lipid phosphorus, and Franey and Amador (1967) for cholesterol and cholesteryl ester. In each case the final volume was 0.4 ml. Protein was measured by the Lowry procedure (1951). Amino sugars were determined with the amino acid analyzer as described below. Total neutral sugars were determined with phenol–sulfuric acid (Montgomery, 1961) with D-mannose as standard. Sialic acid was measured using thiobarbituric acid (Warren, 1959) with *N*-acetylneuraminic acid as standard. A blank citrate eluate not containing enzyme was carried through the procedures for carbohydrate analysis.

For amino acid analysis, samples containing 100–120 μg of purified protein were dialyzed against 0.005 M ammonium carbonate solution (pH 8.6) and then lyophilized. At least two samples of each enzyme preparation were taken for hydrolysis; one was subjected to performic acid oxidation before hydrolysis for cysteic acid determination (Moore, 1963). The oxidized protein was hydrolyzed with HCl (without mercaptoethanol) for 18 hr at 110°. Otherwise hydrolysis was with constant boiling HCl containing mercaptoethanol (1 μl/2 ml) or, in two instances, with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole. With HCl, the sample was degassed and sealed under vacuum and then hydrolyzed for 22 or 40 hr at 110°. In methanesulfonic acid hydrolysis, which yields tryptophan and amino sugars in good yield (Liu and Chang, 1971), the evacuated sample was kept at 110° for 24 hr. The 4-hr procedure for amino acid analysis with a Beckman 120 C analyzer was essentially that described in Beckman Instruments (Palo Alto, Calif.) Bulletin AT-B-033. For analysis of the methanesulfonic acid hydrolysates, the usual short column was replaced by a 20-cm column of PA 35 resin as described by Liu and Chang (1971). Known amounts of glucosamine, galactosamine, mannosamine, and tryptophan were added to the commercially available standard mixture of amino acids for standardization of the procedure.

Results

Specific Activity of Purified Lipase. The isolation of the heparin-released lipase in these studies was somewhat different from our previous preparations (Fielding, 1969). The modifications yielded an enzyme that was considerably more soluble in the absence of detergents and that contained about 2% phospholipid and no detectable (<1%) free cholesterol, cholesterol acyl ester, or triglyceride. The final specific activity (2500–3000 lipase units (micromoles of fatty acid released/hour at 37° with very low density lipoprotein substrate/milligram of protein)) was very similar to that from previous results (Fielding, 1969), *i.e.,* it was not reduced by extraction of lipid under mild conditions. However, extraction of the residual phospholipid with mixtures of ethanol and diethyl ether or chloroform and methanol under the same conditions resulted in complete inactivation and insolubility of the lipase preparation.

Immunodiffusion of Purified Lipoprotein Lipase. The protein prepared by the present procedure gave a single immunoprecipitin line with anti-lipase antiserum; it did not react with rabbit antisera to rat serum lipoproteins or albumin (Figure 1). Addition of 1 μg of these latter antigens (lipoproteins, albumin) in other experiments (not shown) produced detectable reaction with the corresponding antiserum. Thus purified lipase behaved by immunodiffusion as a single enzyme species that was essentially free of plasma lipoproteins and albumin.

Homogeneity of Lipase in Disc Electrophoresis. The disc electrophoresis pattern of purified lipoprotein lipase is shown in Figure 2. In the presence of 0.1% sodium dodecyl sulfate a satisfactory linear plot was obtained between the logarithm of molecular weight and relative migration distance of the specified protein standards. Under these conditions lipase pretreated with 5% detergent migrated with an apparent molecular weight of 37,500 ± 1500 (four experiments). On heavily loaded gels, faint bands corresponding to molecular weights of 72,000 and 145,000 were sometimes seen. Some material was retained at the top of the gel. Lipase not pretreated with detergent as described, but electrophoresed in sodium dodecyl sulfate at 3–5°, showed protein almost entirely in a band corresponding to a molecular weight of about 145,000. Evidence discussed below suggests that the higher molecular weight material represents polymerized forms of a monomeric protein of molecular weight

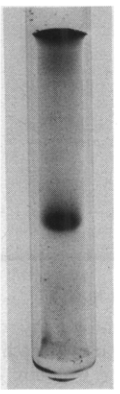

FIGURE 2: Disc electrophoresis pattern of purified lipase in 5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate. Protein was pretreated with 5% detergent in the presence of 0.1 M 2-mercaptoethanol.

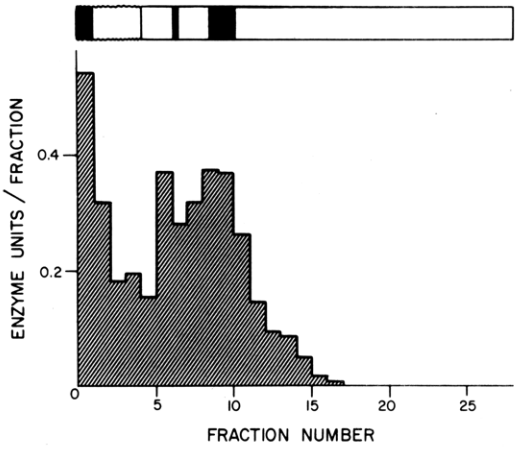

FIGURE 3: Disc electrophoresis of purified lipoprotein lipase on 7% polyacrylamide gel in the absence of detergent. Current was 2 mA and electrophoresis was at 4° for about 2.5 hr. Above, gel after electrophoresis stained with Coomassie Blue; below, lipase activity in 0.2-cm slices of gel immediately after electrophoresis.

37,000. The fraction corresponding to this molecular weight was cut out of the gel and was found to have an amino acid composition without significant differences from the analysis of the total protein applied to the gel. Polyacrylamide gels run in the absence of detergent showed two bands, one diffuse, migrating within the running gel, and a band within the stacking gel. Each band showed corresponding lipase activity when assayed from gel slices (Figure 3). Recovery of lipase activity from gels run at low amperage and 4° was up to 20%. Further lipase activity could be recovered in solution from the top of the stacking gel. It has been previously shown that noncovalent association between protein subunits gives rise to multiple bands on polyacrylamide gels in the absence of sodium dodecyl sulfate, *e.g.*, for serum albumin as described by Hedrick and Smith (1968). We have not attempted to make an independent estimation of molecular weight of these species by the method of these authors, but the rates of migration are compatible with the concept that the faster and slower running gel bands represent low and higher molecular weight forms of lipase.

TABLE I: Amino Acid Composition of Rat Plasma Lipase.

Amino Acid	Moles/10^3 Moles[a] of Amino Acids	Moles/ Subunit
Lysine	58.4 ± 1.3	18
Histidine	13.7 ± 1.6	4
Arginine	62.7 ± 5.8	19
Aspartic acid	81.5 ± 1.1	25
Threonine	58.2 ± 1.2	18
Serine	76.6 ± 5.8	23
Glutamic acid	158.6 ± 5.9	48
Proline	47.1 ± 3.2	14
Glycine	77.8 ± 6.5	24
Alanine	75.5 ± 2.9	23
Half-cysteine	20.6 ± 1.9	6
Valine	62.0 ± 2.1	19
Methionine	20.8 ± 1.5	6
Isoleucine	32.8 ± 2.2	10
Leucine	86.6 ± 6.0	26
Tyrosine	27.3 ± 1.5	8
Phenylalanine	27.3 ± 1.5	8
Tryptophan	13.1	4
(Glucosamine	12.9	4)

[a] Values except for tryptophan and glucosamine are the means ± S.D. of analyses of five different samples of lipoprotein lipase. Experimental values for serine, threonine, cysteic acid, and tryptophan were corrected for losses of 10, 5, 8, and 15%, respectively. Tryptophan and glucosamine values are the means of two separate determinations each from two preparations of enzyme protein.

Molecular Weight of Lipase by Sedimentation Equilibrium. Further information on the minimum molecular weight of the purified lipase was obtained by sedimentation equilibrium studies in the analytical ultracentrifuge. The computed slope of the plot of $1/rdc/dr$ *vs.* Δc was 1.70 ± 0.02 and the calculated mean minimum molecular weight (M_z) was 40,500 for a $\bar{\nu}$ of 0.72 calculated from the chemical composition of this protein by the method of Cohn and Edsall (1943) for amino acids, Gibbons (1966) for the carbohydrate moiety, and Dole and Hamlin (1962) for phospholipids (assuming mol wt 750 for this residue).

Composition of Lipoprotein Lipase. Amino acid analysis of the purified lipase (Table I) revealed no unusual properties of the enzyme or striking features of composition. It contained all of the amino acids commonly found in proteins, none in very small amount, and there was no predominance of apolar residues. Glucosamine was found in HCl as well as methanesulfonic acid hydrolysates. Values for tryptophan and glucosamine are from the latter. Values for other amino acids were in good agreement irrespective of the type of hydrolysis. The data are consistent with a minimum molecular weight of about 36,000 for the purified protein. However, they do not exclude the existence of an even smaller molecular weight of about 17,000, which is the smallest value consistent with near integral values of each of the amino acids.

The carbohydrate composition by weight of purified lipase was: sialic acid (as neuraminic acid) 1.8%; neutral sugars (as mannose) 3.2%; amino sugars (as glucosamine) 1.9%. Values are the means of analyses of two different samples of purified lipase. No galactosamine or mannosamine was detected.

Lipase–Heparin Association. The presence of only small amounts of hexosamine in purified lipoprotein lipase (~4 residues/mol of protein) indicated the absence of a significant endogenous mucopolysaccharide component. Further experiments were undertaken to investigate the possible existence of a complex between the enzyme and the

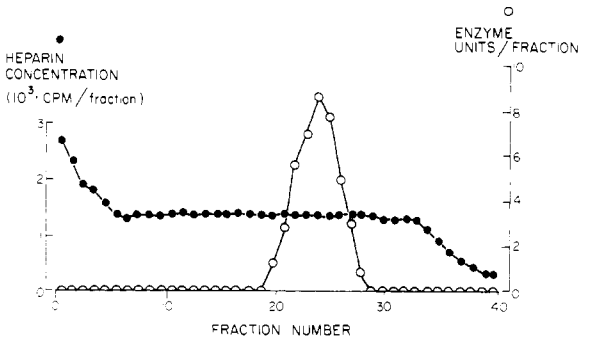

FIGURE 4: Sedimentation of lipase in a linear (5–20% w/v) sucrose gradient in 0.05 M NH_4OH–NH_4Cl and 0.5 mM potassium linolenate (pH 8.3) containing 12 μg/ml of [^{35}S]heparin; 0.1 ml of lipase solution (0.5 mg of protein/ml; 2500 lipase units/mg) was layered on the gradient before centrifugation for 15 hr at 38000 rpm in the SW-39 rotor.

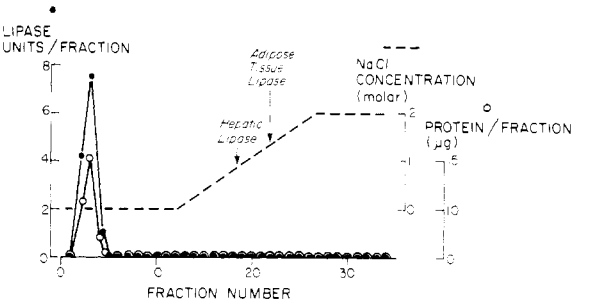

FIGURE 5: Affinity chromatography of lipoprotein lipase, hepatic post-heparin lipase, and adipose tissue lipoprotein lipase on Sepharose 4B–heparin covalent complex. Column: 1.2 × 5 cm. Flow rate: 25 ml/hr at 4°. All lipase samples were run on the same column. Indicated elution points for crude hepatic and adipose tissue lipases represent the activity peaks. In the case of each lipase, washing with 5 M NaCl failed to release further lipase activity from the column.

heparin of post-heparin plasma. Lipase was released with [^{35}S]heparin and purified from post-heparin plasma by the method described. Heparin content during purification is shown in Table II. The purified lipase contained less than one heparin molecule per 1000 molecules of lipase. Unstable heparin–lipase association was investigated by ultracentrifugation of the enzyme on sucrose density gradients containing initially uniform heparin concentrations of from 1 to 50 μg of heparin/ml. Under the conditions shown in Figure 4, binding of 1 mol of heparin/mol of lipase would result in a peak of radioactivity about 20% above the background radioactivity. No indication of heparin–lipase association was obtained under these conditions.

Purified lipase protein was recovered in a yield of about 75% (but with only 25% added lipase activity) in the void volume of an affinity chromatography column of heparin covalently complexed with Sepharose. No further protein was recovered by washing the column with NaCl solutions up to 5 M. Hepatic post-heparin lipase and adipose tissue lipoprotein lipase were adsorbed to the column under the same conditions and eluted in the positions shown in Figure 5.

Discussion

Lipoprotein lipase released into the plasma probably represents the whole lipase activity of the extrahepatic tissues which is involved in the hydrolysis of lipoprotein triglyceride (Fielding and Higgins, 1974). The amount of enzyme released is small. On the basis of the calculated yield from post-heparin plasma, the 400-g rat contains about 50 μg of heparin-released lipase protein, of which about 10 μg can be obtained in highly purified form by the procedure reported here. Tissues such as heart muscle contain several times as much lipoprotein lipase activity in an intracellular (nonreleasable) compartment as is present on the endothelial surface.

The lipase characterized in these studies is evidently distinct from a heparin-released lipase of hepatic origin which shows predominant activity with micellar substrates (Fielding, 1972). An intracellular lipoprotein-activated lipase has been very recently purified from pig adipose tissue by Bensadoun and coworkers (1974). Meaningful comparison between these lipases must await characterization of the tissue enzyme.

Plasma lipoprotein lipase, purified as described, retained about 1.8% residual phospholipids, equivalent to about 1 mol/40,000 g of lipase protein, and also contained small amounts of glucosamine, neutral sugar, and sialic acid. The low hexosamine content of the purified lipase makes it unlikely that the molecule contains any significant endogenous mucopolysaccharide moiety. Results of purification of lipase from post-[^{35}S]heparin plasma and the lack of reaction of this lipase with soluble or matrix-supported heparin suggest that no strong reaction occurs between this lipase and endogenous heparin. Purified lipase in a system containing synthetic lipid substrate and phospholipid and the unique polypeptide cofactor was not stimulated by heparin (Havel *et al.*, 1973). However, during the final purification steps, residual lipoprotein protein, labeled heparin, and the ability of the compound to stimulate lipase activity were simultaneously removed. Lipase activity with high density lipoprotein protein is not stimulated by heparin, but stimulation was found with heparin in the presence of very low density lipoprotein protein (C. J. Fielding, unpublished experiments). It is therefore most likely that the increase in lipase activity induced by heparin in the presence of whole serum (Korn, 1957; Whayne and Felts, 1970) is expressed through the mediation of a plasma component removed during the purification, possibly the β-protein moiety of the substrate lipoproteins.

The molecular weight of purified lipase has been determined by several techniques. A previous study has shown that the isolated enzyme protein stabilized by linolenate

TABLE II: [^{35}S]Heparin Content of Post-Heparin Plasma and Purified Lipase.[a]

Fraction	Protein (mg)	Heparin (μg)	μg of Heparin/mg of Protein
Post-heparin plasma	2040	1910	0.94
Enzyme–substrate complex (after flotation)	0.73	3.2	4.38
Deoxycholate-soluble fraction	0.28	1.9	6.78
Citrate eluate	0.09	<0.002	<0.02

[a] Purification was from a 35 ml plasma sample. Initial specific activity was 0.72 lipase unit/mg of protein. Final specific activity was 2200 lipase units/mg of protein. Heparin content was calculated from the radioactivity of heparin of known specific radioactivity.

had an apparent molecular weight of 72,600 by gel column chromatography (Fielding, 1969). Under these conditions, more than 95% of protein comigrated as a single peak with the assayed lipase activity. In the present report, the treatment of lipoprotein lipase with high concentrations of detergent led to the recovery of a protein species with a molecular weight of about 38,000. Sedimentation equilibrium studies also provided evidence for a low molecular weight lipase species. There is no evidence at present as to which lipase species is present in the presence of triglyceride substrate, although the stability of the predominant enzyme species under the different conditions described may indicate that the dimer is the preferred configuration. It may be relevant that the monomeric molecular weight was obtained only under conditions such that the biological activity of the preparation had been largely lost. However, the identification of more than one active lipase species during gel electrophoresis in the absence of dissociating agents may indicate a reversible equilibrium between the forms, at least in solution. It would be of interest to know the molecular weight of the enzyme at the capillary wall since the natural site of action of the enzyme is at the endothelial membrane rather than in solution. However, an accompanying report (Fielding and Higgins, 1974) indicates that the enzymatic properties of lipoprotein lipase at the membrane site may be similar to those of the soluble lipase.

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