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Lipoprotein Lipase. Isolation and Characterization of a Second Enzyme Species from Postheparin Plasma†

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ABSTRACT: A lipoprotein lipase species (mol wt 69 250) has been isolated from rat postheparin plasma, which differs from the low-molecular-weight species previously characterized in its amino acid composition and hexosamine content, and in its lower affinity for triglyceride-rich lipoprotein substrates. However, both enzymes are activated by the same coprotein (C-terminal glutamic acid, apo-C-2) from human very low density lipoprotein and have a similar specificity for lipid esters. Neither purified enzyme is activated by heparin. Both are inhibited by molar sodium chloride. Both enzyme species can be recovered from the same plasma samples. The possible relationship of these proteins to the different functional lipoprotein lipase activities of muscle and adipose tissues is discussed.

Lipoprotein lipase at the vascular surface of different tissues is functionally heterogeneous. In experiments with perfused heart (Fielding and Higgins, 1974) and adipose tissues (Fielding, 1976), kinetic studies of the membrane-supported lipase suggested the presence of enzyme sites with high and low affinities, respectively, for reaction with natural lipoprotein substrates. The lipase with high substrate affinity, released by heparin from the perfused heart, has been isolated and characterized from postheparin plasma (Fielding et al., 1974). The purpose of the present study was to identify and isolate from postheparin plasma a component corresponding to the low affinity lipoprotein lipase species.

Experimental Section

Lipoprotein Preparation. Very low density lipoproteins were isolated from the plasma of male Sprague-Dawley rats (300–350 g) by ultracentrifugal flotation (Havel et al., 1955). Plasma containing 0.1% Na_2EDTA[1] (pH 7.4) was centrifuged at $1.6 \times 10^5 g$-min and the floating lipoprotein material (S_f, flotation index, > 400) (Dole and Hamlyn, 1962) was discarded. The infranatant fraction was centrifuged for $4.86 \times 10^6 g$-min and the floating triglyceride-rich lipoprotein, after recentrifugation under the same conditions, was used as lipase substrate in the experiments described below.

Chylomicrons were prepared from rat intestinal lymph obtained from animal donors bearing a cannula in the mesenteric lymph duct and in the duodenal lumen (Fielding and Higgins, 1974). Synthetic triglyceride–lecithin dispersion (Intralipid, Cutter Laboratories, Oakland, Calif.) (2.5% w/v triglyceride) was infused at a flow rate of 2 mL/h into the duodenum. Lymph collected for up to 18 h in ice-cooled tubes was centrifuged at $9.5 \times 10^4 g$-min to remove the largest particles, then the infranatant fraction was recentrifuged at 1.6×10^5 g-min to float the major chylomicron fraction. This was recentrifuged under the same conditions. Triglyceride-depleted plasma was prepared by removal of very low density lipoprotein ($d < 1.006$ g cm^{-3}) from whole plasma. The infranatant solution, containing residual plasma proteins concentrated two- to threefold (Fielding and Higgins, 1974) was dialyzed against 500 volumes of Krebs–EDTA buffer (pH 7.4).

Preparation of Lipoprotein Polypeptides. Human very low density lipoprotein obtained by centrifugation from the plasma of normal donors was delipidated with mixtures of diethyl ether and ethanol (Shore and Shore, 1969). Low-molecular-weight polypeptides were separated from total apoprotein by gel filtration on Sephadex G-150 (Pharmacia, Piscataway, N.J.) in

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[1] Abbreviations used: Na_2EDTA, ethylenediaminetetraacetic acid, sodium salt; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane.

7 M urea, followed by DEAE-cellulose chromatography as previously described (Shore and Shore, 1969). Component isolated polypeptides (whose amino acid analysis did not differ significantly from those previously reported (Shore and Shore, 1972)) were dialyzed against distilled water and stored at −70 °C.

Amino Acid Analysis. Samples of purified lipoprotein lipase (23–33 μg of plasma lipase protein), adsorbed to 0.2 mL of calcium phosphate gel (5.7 mg dry wt), were washed twice with 5 mL of distilled water before hydrolysis. Two 90-μg samples of bovine plasma albumin on calcium phosphate gel, one sample in solution in the absence of gel, and reagent blanks were analyzed under conditions identical with those used for the lipase samples for assessment of recoveries of amino acids. Hydrolysis of proteins with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Pierce Chemical Co., Rockford, Ill.) was carried out in evacuated tubes at 110 °C for 40 h. Calcium phosphate was removed by adjustment of the hydrolysate pH to 7.8 and centrifugation. The insoluble gel was washed three times with 100 μL of distilled water adjusted to pH 7.8 with sodium hydroxide. The combined supernatant and precipitate washings were adjusted to pH 2.2 for amino acid analysis with a Beckman 120C analyzer essentially as described in Beckman Instruments (Palo Alto, Calif.) publication A T-B-033 but with the short column modification described by Liu and Chang (1971). Reagent blanks contained no amino acids. The composition of bovine plasma albumin carried through the gel step corresponded within experimental error to that determined directly and to the analysis reported by others (Putnam, 1965) after application of extrapolated correction factors for destruction of serine (8%), cysteine (18%), methionine (6%), and tryptophan (12%).

Molecular Weight Determination. Disc electrophoresis in 5% polyacrylamide gel was carried out as described by Dunker and Rueckert (1969). Isolated lipase protein was heated at 100 °C for 20 min in the presence of 1–5% w/v sodium dodecyl sulfate and 0.1 M 2-mercaptoethanol. Addition of 4 M urea was without effect on protein migration. After completion of the run, gels were simultaneously fixed and stained in 0.25% w/v Coomassie brilliant blue in acetic acid–methanol–water (10:45:45 v/v) at 70 °C for 45 min and then destained in acetic acid–methanol–water (5:5:90 v/v) under the same conditions (Laemmli, 1970). In some experiments gels were fixed with 10% trichloroacetic acid and stained at room temperature with Coomassie blue. The patterns obtained with each system were identical. Protein mobility, a function of molecular weight, was determined simultaneously with that of protein standards of known molecular weight as described previously (Fielding et al., 1974) including rat plasma high-affinity lipoprotein lipase (mol wt 37 500).

Assay of Lipoprotein Lipase Activity. The specific activity of purified lipase preparations was determined using triolein substrate dispersed with synthetic dioleyllecithin (Serdary, London, Ontario) (10% w/w) by sonic irradiation as previously described (Fielding, 1973). Lipase activity was assayed as the rate of production of unesterified fatty acid at pH 8.3 and 37 °C in the presence of 3% w/v albumin, 10% v/v recalcified citrated rat plasma, and 50 mM Tris-HCl buffer, pH 8.3. Free fatty acid was determined titrimetrically using the Radiometer ABU-11 autoburet with 0.05 mM tetramethylammonium hydroxide. Protein was determined, after hydrolysis, with ninhydrin. Palmitoyl-CoA hydrolase activity of purified lipase preparations was determined as described by Jansen and Hulsmann (1973). Lipase activity with natural lipoprotein substrates (chylomicrons, very low density lipoproteins) was determined at pH 7.4 in the presence of Krebs–Ringer bicarbonate buffer, 3% w/v albumin (pH 7.4), and 10% v/v triglyceride-depleted plasma. Catalytic rate and apparent Michaelis constants (k_{cat} and $K_{m(app)}$) for purified lipase were determined from the double-reciprocal plots ($1/v$ vs. $1/s$) fitted by least-squares analysis for at least six experimental points in duplicate. The rate of phospholipase activity of purified lipoprotein lipases with natural lipoprotein substrates was determined using chylomicrons labeled in the phospholipid moiety. [^{32}P]orthophosphate (Amersham/Searle, Chicago, Ill.) (50 μCi/mL) was passed into the duodenal lumen of animal donors receiving triglyceride above. Chylomicrons collected in the intestinal lymph were purified as before. The specific activity of the major chylomicron phospholipids (lecithin and phosphatidylethanolamine) was determined after Folch extraction of total chylomicron lipids and separation of component phospholipid classes by thin-layer chromatography on silica gel plates developed in chloroform–methanol–water (65:35:4 v/v) (Fielding and Fielding, 1976). Since in this system lecithin and lysophosphatidylethanolamine are not well separated, lecithinase was determined as the rate of production of labeled lysolecithin, and the rate of hydrolysis of phosphatidylethanolamine and sphingomyelin as the loss of lipid radioactivity from the corresponding lipid species.

Results

Purification of High- and Low-Affinity Lipases from Postheparin Plasma. Plasma was obtained from rats exsanguinated 5 min after intravenous injection of heparin (Upjohn, Kalamazoo, Mich.) (100 IU/kg of body wt). The plasma contained 30–45 lipase units/mL (μmol of unesterified fatty acid released h^{-1} from triolein substrate at pH 8.3, 37 °C).

Step 1: Flotation of Lipase–Substrate Complex. Plasma lipoprotein lipases were complexed with triglyceride dispersion (final concentration 5 mg/mL) by incubation at 37 °C for 7 min (Fielding et al., 1974). Each 10 mL of activated plasma, immediately cooled in ice, was layered under 20 mL of 0.05 M NH_4OH–NH_4Cl buffer, pH 8.3, and centrifuged in the 30 rotor of the Spinco L3-50 ultracentrifuge for 30 min at 4 °C. The floating packed lipid and associated opalescent layer (10 mL total volume) were recovered by tube-slicing. The infranatant fraction (containing the bulk plasma proteins) contained 15.7 ± 4.1% of the original total plasma lipase content (mean ± SD, range 9.6–22.3%, ten experiments). The bulk of this lipase activity was a salt-stable species probably representing hepatic postheparin triglyceride lipase (Fielding, 1972). The lipid-rich fraction was recentrifuged under the same conditions and the supernatant layer (recovered in 1–2 mL) was separated from the intermediate opalescent zone (4–5 mL). The packed floating lipid was recentrifuged under the same conditions and the entire infranate pooled with the opalescent fraction. Recovery in this pooled material was 33.2 ± 9.6% (6 experiments) of total plasma lipase activity, purified 75–100-fold from plasma at this step. The final lipid-rich supernatant, containing the remainder of the floating lipase (about 50% of total plasma activity), was retained for purification of plasma high-affinity lipase by the method previously described (Fielding et al., 1974).

Step 2: Delipidation of Lipase in Pooled Infranatant. The pool from step 1 was mixed with 0.1 volume of 10% high-molecular-weight dextran (Pharmacia, Dextran T-500) in 0.05 M ammonia buffer (pH 8.3). The mixture was added dropwise to 7 volumes of acetone cooled in ice. The coprecipitate of protein and dextran was filtered, washed at room temperature

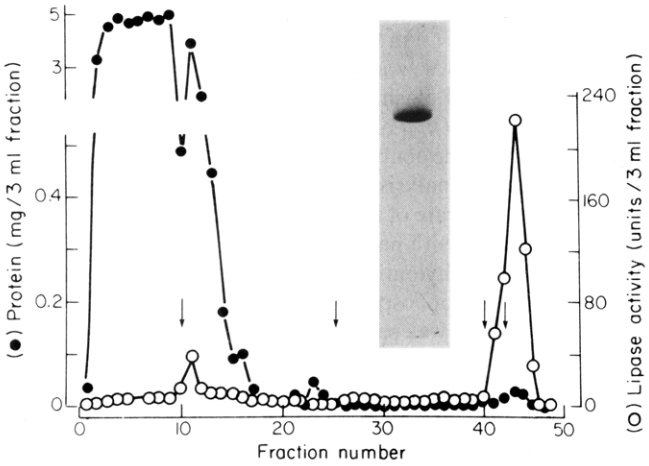

FIGURE 1: Chromatography of low-affinity plasma lipoprotein lipase on heparin–Sepharose. (↓) Point of addition of buffers containing increasing concentrations of NaCl (0.5, 0.65, 0.8, and 1.3 M NaCl from left to right of the figure). Protein concentrations were determined with recrystallized bovine serum albumin standard and lipase activities were determined at pH 8.3 with synthetic triglyceride dispersion activated with rat plasma as described under the Experimental Section. (Insert) Polyacrylamide gel electrophoresis of low-affinity lipoprotein lipase isolated from postheparin plasma.

TABLE I: Amino Acid Composition[a] of Lipoprotein Lipase Species.

Amino Acid	Low Affinity Lipase[b]	High Affinity Lipase[c]
Lys	81.7 ± 4.8	58.4 ± 1.3
His	21.8 ± 1.5	13.7 ± 1.6
Arg	48.0 ± 2.2	62.7 ± 5.8
Asp	103.2 ± 7.0	81.5 ± 1.1
Thr	49.9 ± 1.5	58.2 ± 1.2
Ser	85.4 ± 5.7	76.6 ± 5.8
Glu	102.3 ± 3.6	158.6 ± 5.9
Pro	59.3 ± 3.3	47.1 ± 3.2
Gly	62.6 ± 5.7	77.8 ± 6.5
Ala	65.0 ± 2.5	75.5 ± 2.9
½-Cystine	27.0 ± 0.9	20.6 ± 1.9
Val	63.3 ± 1.6	62.0 ± 2.1
Met	15.3 ● 3.0	20.8 ± 1.5
Ile	40.2 ± 1.4	32.8 ± 2.2
Leu	85.5 ± 1.8	86.6 ± 6.0
Tyr	30.9 ± 1.6	27.3 ± 1.5
Phe	45.4 ± 2.1	27.3 ± 1.5
Trp	14.0 ± 1.5	13.1
Glucosamine	18.8 ± 2.4	12.9

[a] In moles per 10^3 moles of amino acids. Values are means ± SD of three analyses of different enzyme preparations for 23–33 μg of low affinity lipase protein. [b] Low affinity lipase (mol wt 69 250) from heparin–Sepharose affinity chromatography. [c] High affinity lipase (mol wt 37 500); values are taken from Fielding et al. (1974). All analyses were standardized with the same standard mixture of amino acids.

with 200 mL of acetone, and then successively with 100 mL of heptane, 100 mL of 50% ethanol–diethyl ether, and finally with 200 mL of diethyl ether. Remaining solvent was removed under vacuum at room temperature. The precipitate from 100–150 mL of original postheparin plasma was dispersed in 40 mL of 0.05 M ammonia buffer, pH 8.3. Insoluble material was removed by filtration at 4 °C through glass fiber paper and the paper washed with 15 mL of 0.005 M barbital buffer, pH 7.0, followed by 15 mL of the same buffer containing 0.5 M NaCl. Recovery at this stage was about 70% of activity from step 1. Lipase was purified 100–150-fold from plasma at this step.

Step 3: Chromatography on Heparin–Sephadex Covalent Complex. Adipose tissue and mammary tissue lipoprotein lipases have been shown to form stable complexes with heparin bound to Sepharose 4B (Pharmacia) via the cyanogen bromide reaction (Egelrud and Olivecrona, 1972; Bensadoun et al., 1974). Plasma high affinity lipase did not react under these conditions (Fielding et al., 1974). The lipase fraction separated from high affinity lipase in step 1 and delipidated in step 2 was applied to a column (1.2 × 5 cm) containing Sepharose 4B covalently complexed with heparin (Wilson Laboratories, Chicago, Ill.) (Iverius, 1971). The column was washed with 35 mL of 0.5 M NaCl–0.005 M barbital–sodium barbiturate buffer (pH 7.0). Only a small proportion of applied lipase (5–10% of added activity) was recovered in this fraction. The column was then washed with 0.65 M NaCl in the same barbiturate buffer until the eluate contained less than 0.5 μg of protein/mL (detectable by the fluorescamine reaction, Udenfriend et al., 1972) (Figure 1). Barbital buffer containing 0.8 M NaCl (10 mL) was passed through the column and finally 15 mL of 1.3 M NaCl in the same buffer. A single peak of lipase of constant specific activity across the peak was released. This activity was further characterized in terms of its molecular and chemical properties as described below. Recovery through the column procedure was about 25–40% of applied activity. Final yield from total plasma lipolytic activity was about 8%. Final specific activity was 10 000–14 000 lipase units/mg of protein, representing a purification of about 17 000–20 000-fold from whole postheparin plasma.

An abbreviated method was developed to permit isolation of this lipase fraction in improved yield when simultaneous isolation of the high-affinity lipase fraction was not required. The first centrifugation of the lipase–triglyceride complex was followed immediately by delipidation and chromatography on Sepharose–heparin as described above. Under these conditions, yield of lipase eluted with 1.3 M NaCl–barbiturate buffer was 12–16% of original total postheparin lipolytic activity. Specific activity of the product was within the range obtained for the original procedure.

Plasma high affinity lipoprotein lipase was isolated by further flotation, detergent fractionation, delipidation, and chromatography as previously described (Fielding et al., 1974) using the final lipid–lipase floating complex discarded in step 1 as starting material. In some experiments detergent fractionation was with [^{3}H]deoxycholate (New England Nuclear, Boston, Mass.). The product retained <0.05% of detergent concentration at the solubilization step, representing an assayed concentration of <1.0 μg/mL deoxycholate. In other experiments solubilization of the lipase–lipid complex was achieved without deoxycholate, with either 0.5 mM potassium linolenate or with ammonia buffer (pH 8.3) after delipidation with ethanol–diethyl ether (2:1 v/v at −20 °C). The product in each case had a final specific activity of 3400–4000 lipase units/mg of protein, similar to that previously reported (Fielding et al., 1974).

Molecular Weight of Lipoprotein Lipase Species. The lipase species previously isolated from postheparin plasma had a molecular weight of about 37 500 by polyacrylamide gel electrophoresis, of which a 34 200-dalton moiety represented protein; the isolated protein also contained 6.9% by weight of carbohydrate and 1 mol/mol protein of tightly bound phospholipid (Fielding et al., 1974). The lipase species now purified from plasma by heparin–Sepharose chromatography migrated

TABLE II: Activation and Inhibition of Plasma Lipoprotein Lipases.

Reagent	% of Plasma-Activated Hydrolysis Rate[a]	
	Low Affinity Lipase[b]	High Affinity Lipase[c]
Basal activity (− plasma)	5	7
M-NaCl	10	10
Protamine (1 mg/mL)	19	19
Heparin (10 μg/mL)	93	96
Apo-C-1 (1.0 μg/mL)	5	3
Apo-C-2 (1.0 μg/mL)	89	86
Apo-C-3-1 (1.0 μg/mL)	5	5
Apo-C-3-2 (1.0 μg/mL)	5	3

[a] Values are means of duplicate determinations of activity at pH 8.3 and 37 °C in the presence of an initial concentration of 2.0 μmol/mL triolein substrate as described under the Experimental Section. Assays with M-NaCl, protamine, and heparin were carried out in the presence of 10% v/v plasma. [b] Low-affinity lipase (mol wt 69 250) isolated by heparin-Sepharose chromatography. [c] High-affinity lipase (mol wt 37 500) isolated as described by Fielding et al. (1974). K_m values for low- and high-affinity lipoprotein lipases under these assay conditions were 0.25 and 0.09 mM triglyceride, respectively.

as a single protein species with an estimated molecular weight of 69 250 ± 650 (five determinations) (Figure 1, insert). The molecular weight of the other lipase fraction run at the same time was not significantly different from that previously reported.

Analysis of Amino Acids and Amino Sugars. Preliminary experiments indicated that dialysis of purified lipoprotein lipases against a low-salt medium, required before concentration or lyophilization from heparin-Sepharose for amino acid analysis, resulted in the loss of more than 75% of enzyme protein from solution. The following procedure, which is of general application, permitted complete recovery of enzyme protein free of salts and ninhydrin-reactive buffer anions. Total protein and lipase activity was adsorbed to calcium phosphate gel, as described under the Experimental Section. There was no detectable enzyme activity or protein in the supernatant solution after centrifugation of the gel. Hydrolysis, by the modified method described, was carried out on the two purified lipase species. As shown in Table I the high- and low-affinity lipase fractions from postheparin plasma showed a number of significant differences in amino acid composition. Plasma low affinity lipase also contained a greater content of hexosamine. Only glucosamine (and no galactosamine or mannosamine) was detected in these experiments.

Activation and Inhibition of Lipoprotein Lipases. The different enzyme species reacted similarly with several common activators and inhibitors of lipoprotein lipase when synthetic triglyceride dispersion was the substrate. Both high- and low-affinity enzymes were activated by plasma and were inhibited to a similar extent by M-NaCl (Table II). Heparin did not significantly activate any of the purified preparations. Protamine inhibited both pure enzyme proteins. Both the activities isolated from postheparin plasma were activated for reaction with synthetic triglyceride substrate only with apo-C-II (C-terminal glutamic acid) (Shore and Shore, 1972) of the major polypeptide components of human triglyceride-rich lipoprotein.

Palmitoyl-CoA hydrolase activity has been proposed as a property by which the activities of different plasma lipases can be distinguished (Jansen and Hulsmann, 1973). Palmitoyl-CoA hydrolase activity was 7.9 and 6.9% of triglyceride hydrolase activity for high- and low-affinity lipases, respectively.

Kinetics of Reaction with Natural Lipoprotein Substrates. Previous research had indicated distinct kinetic properties for lipoprotein lipase at the vascular surface of perfused heart and adipose tissues (Fielding, 1976). Kinetic constants for lipase species in the present experiments were determined at pH 7.4 with very low density lipoprotein and chylomicrons. As shown in Table III, the K_m value for low-affinity lipase from heparin-Sepharose was significantly greater than that for the high affinity species. However, both lipases showed a greater rate of activity with chylomicron than with very low density lipoprotein triglyceride. The characteristic properties of low-affinity lipase were unchanged in the presence of low concentrations of deoxycholate (1 μg/mL). Both enzymes showed minor phospholipase activity with the lecithin and phosphatidylethanolamine moieties of chylomicrons labeled with [^{32}P]orthophosphate. Relative hydrolysis rates were not significantly different for the two lipases and the lecithinase/triglyceride hydrolase ratio was 0.037 ± 0.012 for low-affinity lipase and 0.032 ± 0.012 for the high-affinity species (four experiments). Corresponding values for phosphatidylethanolamine hydrolase were 0.031 ± 0.013 and 0.024 ± 0.014. Lipoprotein lipase showed no detectable sphingomyelinase activity with chylomicron sphingomyelin. The low rates of phospholipase activity reported here are similar to those found with synthetic lipid substrates (Fielding and Fielding, 1976).

Effects of Deoxycholate on Binding to Heparin-Sepharose. Deoxycholate at concentrations up to 100 μg/mL had no effect on the binding of the low-affinity lipase species to heparin-Sepharose and, when purification was carried out by affinity chromatography in the presence of detergent, there was no increase in the proportion of total lipase activity not absorbed by heparin-Sepharose (7.6 ± 2.7% vs. 8.2 ± 2.0% in the absence of detergent).

TABLE III: Apparent Kinetic Constants of Plasma Lipoprotein Lipases.[a]

Enzyme Species	Chylomicrons		Very Low Density Lipoproteins	
	$k_{cat} \times 10^3$ (min^{-1})	$K_{m(app)}$ (mM)	$k_{cat} \times 10^3$ (min^{-1})	$K_{m(app)}$ (mM)
Low affinity lipase[b] (69 250)[c]	5.6 ± 0.3	0.29 ± 0.04	3.8 ± 0.3	0.26 ± 0.03
High affinity lipase[d] (37 500)[c]	3.6 ± 0.1	0.08 ± 0.02	1.8 ± 0.2	0.06 ± 0.02

[a] Values are means ± SD (three experiments). [b] With 1.3 M NaCl eluate from heparin-Sepharose. [c] Molecular weight in parentheses. [d] Citrate eluate from calcium phosphate. Kinetic constants were determined by assay at pH 7.4 and 37 °C. Apparent Michaelis constant ($K_{m(app)}$) is expressed in terms of lipoprotein triglyceride content. Catalytic constant (k_{cat}) was determined from extrapolated maximal reaction velocity (V) and enzyme protein concentration in moles (E) as $k_{cat} = V/E$.

Discussion

Although a number of lipolytic and esterolytic activities are released into plasma by heparin (Shore and Shore, 1961; Greten et al., 1969; Fielding, 1972), it has been considered that lipoprotein lipase activated by apo-C-II and inhibited by inorganic salts represented a single enzyme species. Major differences in the kinetic properties of lipoprotein lipase active in perfused heart and adipose tissues have been described (Fielding, 1976). However, in these experiments it was not possible to distinguish effects due to the lipase molecule itself and to the nature of its membrane binding sites. In fact, the present study provides evidence for the first time that such a difference in properties is due at least in part to differences in the enzyme molecules concerned, and that postheparin plasma contains two apo-C-II activated lipoprotein lipases, with different molecular weights and chemical composition. The low-molecular-weight, high affinity enzyme probably represents the enzyme functional at the vascular surface of the perfused heart (Fielding and Higgins, 1974) and perhaps other muscle tissues. The second, low-affinity lipase, described here for the first time, is closely similar, at least in kinetic properties, to a major fraction of adipose tissue lipase. It therefore appears likely that this lipase activity is released from adipose tissue. This enzyme has a molecular weight of 69 250 and lipases of similar size have been obtained from pig adipose tissue and human postheparin plasma (Bensadoun et al., 1974; Augustin et al., 1976), although chemical analyses of the former are not yet available. Neither the purification, specific activity, heparin affinity, nor kinetic constants of low-affinity lipase were affected by deoxycholate used in the preparation of high-affinity species whose properties were independent of the method of solubilization. However, in spite of differences in affinity for natural lipoprotein substrates, which may be related to the different physiological roles of the lipase in muscle and adipose tissues (Fielding, 1976), numerous similarities between low- and high-affinity lipoprotein lipases indicate a relationship between them. The active site properties of these enzymes are closely similar. Both are activated exclusively by apo-C-II polypeptide. Both forms show a similar spectrum of substrate specificities, with major activity with neutral glyceride and only minor reaction with phospholipids and soluble esters. Finally, antibodies prepared against plasma lipoprotein lipase, purified by the method developed for high-affinity lipase, showed at least partial cross-reactivity by precipitation of adipose tissue lipoprotein lipase (Yasuoka and Fujii, 1971). It thus appears likely that the major functional differences between these enzyme forms lie in their adsorption sites, concerned with substrate affinity, and that their hydrolytic sites are more closely related. Such a relationship could be derived, for example, by posttranslational cleavage (Hershko and Fry, 1975) or through gene duplication, in a manner analogous to that proposed for the high- and low-molecular-weight forms of acetylornithine transaminase (Billheimer and Jones, 1974). It is interesting that in this case also a major difference between the forms lies in their substrate affinity, expressed in terms of low and high apparent Michaelis constants. The present study does provide further evidence to support the concept of tissue-specific lipoprotein lipase activities, originally suggested on the basis of perfusion studies, by isolation of two such species from postheparin plasma. These may be important in regulating clearance of triglyceride fatty acid by different tissues under rate-limiting conditions of substrate concentration.

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