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Lipoprotein Lipase Activity in Bovine Aorta[1] (38696)

PAUL E. DICORLETO[2] AND D. B. ZILVERSMIT[3]

Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853

Lipoprotein lipase is thought to be involved in the control mechanisms of the uptake of chylomicrons from serum into adipose tissue (1, 2). Recently, Zilversmit has proposed that lipoprotein lipase in the arterial wall may be linked to atherogenesis (3). The enzyme is postulated to act on triglyceride-rich lipoproteins at the wall of the artery, forming a cholesterol- and cholesteryl ester-rich remnant that might be incorporated into the arterial intima.

Korn originally characterized lipoprotein lipase, which hydrolyzes triglycerides to glycerol and free fatty acids when the lipid substrate is associated with a lipoprotein or a serum-activated artificial emulsion (4). Lipoprotein lipase (E.C. 3.1.1.3) has since been identified in several mammalian tissues, including adipose, lung, and heart (5, 6). Commonly used substrates in the study of this lipase are dog chylomicrons (7, 8), Ediol (9), and emulsions stabilized with Triton X-100 (10) or phospholipid (11). Many of the characteristics of lipoprotein lipase vary with source of enzyme and type of substrate emulsion; however, some common features of the activity do exist and include: an inhibitory effect of protamine sulfate and of high concentrations of sodium chloride, a stimulating effect of low concentrations of heparin or Ca^{2+}, a pH optimum between 8.0 and 8.6, and an absolute requirement for serum as an activator of artificial substrate emulsions.

Lipoprotein lipase has been identified in the aortas of rat (4), rabbit (12), and pig (13, 14); however, the studies have been primarily histochemical in nature or have employed substrates which contain partial glycerides as well as triglycerides. Thus, the measured lipase could not be distinguished from monoglyceride lipase or other lipases that are known to be present in tissues. It should also be noted that in not one of these previous studies has the lipolytic activity been identified with certainty as lipoprotein lipase.

The purpose of the present study is to identify and partially characterize lipoprotein lipase from a readily available and substantial source of artery—bovine aorta—and to determine the localization of the enzyme by a sectioning of the arterial wall.

Experimental Section. Materials and Methods. I. Preparation of enzyme and sectioning of aorta. Fresh bovine aorta was obtained from a local abattoir and maintained at 4° thereafter. Extraneous fat was dissected from the outside of the vessel. The aorta was then slit longitudinally and pinned to a cork board with the intima facing upwards. Blood was removed by gentle rinsing with 0.9% sodium chloride. The innermost layer of the aorta, endothelium plus some intima, was then carefully scraped with a razor onto a Teflon spatula and rinsed with saline into a vial. This sample was labeled section I. The next layer, section II, approximately 0.5 mm in thickness, was peeled with scissors and forceps from the pinned vessel and placed in saline. This process was repeated to obtain a third layer, section III, which was still essentially free from the fat and adventitia of the outer portion of the artery. Unless otherwise specified, section I and section II combined were chosen as enzyme source.

A 10% homogenate of the tissue sections was prepared by finely mincing the

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[3] Career Investigator of the American Heart Association.

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sample into ice cold physiological saline, followed by maceration on ice with the Willems Polytron (Type PT 10 OD, Number 2703, Brinkmann Instruments, Westbury, NY) for 20 sec at high speed. The homogenate was then centrifuged at 800 *g* for 30 min at the same temperature. The supernatant, 3–4 mg protein/ml, was employed in subsequent assays. Protein determinations were carried out by the method of Lowry *et al.* (15). The homogenate was most stable when stored at 4° in the presence of heparin (10 units/ml), losing only 10% activity in 2 days. The whole tissue lost approximately 90% of its activity when stored at −20° for 3 wk.

II. Assay system. The triolein substrate emulsion was prepared by a slight modification of the method of Schotz and Garfinkel (11). Glycerol tri-[^{14}C]-oleate was used instead of [^{3}H]glyceryl trioleate, and the total concentrations of triolein and egg phosphatidylcholine were reduced to one-third.

Phosphatidylcholine was isolated from egg yolk and purified by the slightly modified method of Singleton *et al.* (16). Activating plasma was added in varying amounts to individual tubes. Each assay contained 0.3 ml substrate emulsion with 4.23 mg triolein (Sigma grade, Sigma Chemical Co., St. Louis, MO), 0.5 mg [1-^{14}C]-triolein (0.19 μCi) (Dhom Products, Ltd., North Hollywood, CA), 0.24 mg phosphatidylcholine, and 5% bovine serum albumin (crystallized, Miles Laboratories, Inc., Kankakee, IL) in 0.53 *M* Tris-Cl buffer pH 8.2;[4] 0.1 ml heated[5] rat plasma (1.0 mg EDTA/ml) or saline; 0.4 ml enzyme homogenate; and 0.2 ml additive or buffer. The substrate emulsion was incubated with plasma for 10 min at 37° before the addition of enzyme. The assay was routinely run for 1 hr at 37° in a shaking constant-temperature bath. The reaction was halted by the addition of 6 ml of the solvent system (methanol:chloroform:heptane, 1.45:1.25:1 v/v/v) in the Belfrage and Vaughan method (17) of oleic acid extraction. The "carbonate buffer" (0.3 ml of potassium borate–potassium carbonate (0.25 *M*)) was then quickly added, the tubes vortexed, capped, and centrifuged at 600 *g* for 10 min at 25°. The radioactivity in an aliquot of the upper layer was determined by liquid scintillation counting in Bray's solution (18). In zero time controls, saline was employed in place of homogenate. Partitioning corrections were made on the basis of controls containing a substrate emulsion as above, but with carrier oleic acid (4.6 mg/ml, Applied Science Laboratories, Inc., State College, PA) and ^{14}C-labeled oleic acid (Dhom Products, Ltd., North Hollywood, CA) and no enzyme or labeled triolein. Effects of each additive—activators or inhibitors—on partitioning, quenching, and zero time values were determined.

Results. The release of fatty acid from triolein of a lecithin-stabilized emulsion was found to be linear for at least 2 hr at 37°, as shown by Fig. 1. The maximum extent of hydrolysis was never greater than 1% of the total substrate present. Figure 1 also shows the release of ^{3}H-glycerol from glyceryl-labeled triolein under identical conditions. The nonlinearity is most likely due to a build-up of partially hydrolyzed triolein molecules, mono- and di-glycerides before free glycerol accumulates. The activity was also found to be linear with respect to volume of homogenate, i.e., concentration of enzyme (Fig. 2). A Michaelis-Menten plot of velocity vs triolein concentration shows the usual saturation kinetics (Fig. 3). It is evident that the standard assay (0.3 ml of emulsion) is done under conditions of zero order kinetics. The apparent K_m of the enzyme is 1 m*M* triolein. The effect of varying pH is shown in Fig. 4. The optimum pH of 8.6 agrees with that observed for adipose tissue lipoprotein lipase (19).

The arterial lipoprotein lipase showed a definite requirement for serum or plasma activation of the artificial emulsion. The basal lipolytic activity of rat plasma, how-

[4] The pH of 8.2 was adopted to conform with the assay of Schotz and Garfinkel (11). At this pH, lipolytic activity is about 15% less than at pH 8.6 (see Fig. 4).

[5] Plasma was heated to 56–60° for 1 hr. Temperatures above 60° cause precipitation of the plasma proteins.

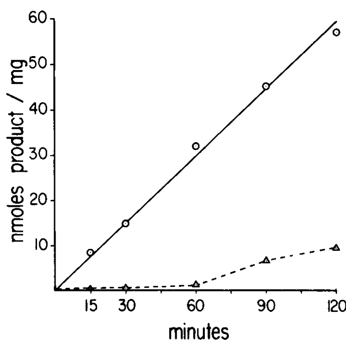

FIG. 1. Time course of hydrolysis of triolein by arterial lipase. The release of the two products [^{14}C]-oleic acid (-⊙—⊙-) and [^{3}H]-glycerol (-△--△-) were measured under identical conditions. Ordinate expressed per mg protein.

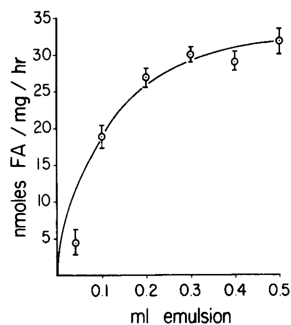

FIG. 3. The effect of triolein concentration on the specific activity of arterial lipase. The concentrations of all components of the substrate emulsion other than the triglyceride were kept fixed. The triolein concentration in the substrate emulsion was 1.59 m*M*.

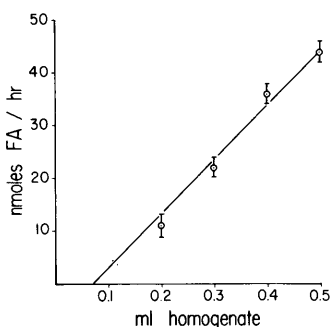

FIG. 2. The rate of hydrolysis of triolein as a function of enzyme concentration. Varying amounts of tissue homogenate and saline were added to the incubation mix to maintain a constant volume of 1.0 ml. The protein concentration of the homogenate was 3.3 mg/ml.

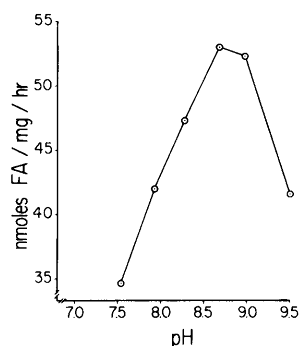

FIG. 4. The effect of pH on the hydrolysis of triolein by arterial lipase. Aliquots of a single substrate emulsion were adjusted to the various pH values by the addition of HCl or Tris. Ordinate expressed per mg protein.

ever, is quite high (900 nmoles FA/hr/ml), and we therefore initially encountered the undesirable situation of measuring the arterial enzyme in the presence of a much more active plasma lipase. The possibility also existed that apparent arterial lipolytic activity was in fact a result of activation of the plasma lipase by some factor in the tissue homogenate. The difficulty was overcome by heating the plasma for 1 hr at 56–60°. The lipolytic activity of plasma was destroyed while its activating properties were retained. In repeated experiments, examples of which are shown in Table I, the activation of lipoprotein lipase by 10% plasma was 20- to 30-fold. Considerable but suboptimal activation was observed with 0.5% plasma.

Inhibition studies have often been used to determine whether or not lipolytic activity is due to lipoprotein lipase. A potent inhibitor of lipoprotein lipase from several sources is protamine sulfate (1, 10). As shown in Fig. 5, bovine arterial lipoprotein lipase was inhibited over 50% by protamine sulfate at low concentrations (1.0 mg/ml) and nearly totally inhibited at higher concentrations (3.0 mg/ml). High concentrations of NaCl also reportedly inhibit lipoprotein lipase (1, 5). The bovine arterial enzyme was inhibited 85% by 1.4 *M* NaCl (Fig. 5). The hormone-sensitive lipase from various sources has been shown to be

TABLE I. EFFECT OF VARIOUS ACTIVATORS ON BOVINE AORTA LIPOLYTIC ACTIVITY.

Addition to Assay	nmoles FA released/mg protein/hr	
	Expt. I	*Expt. II*
No plasma	1.2	0.9
10% heated plasma	22	29
Plasma + Ca^{2+} (10 m*M*)	26	—
Plasma + Ca^{2+} (20 m*M*)	35	—
Plasma + heparin (1 units/ml)	—	30
Plasma + heparin (10 units/ml)	—	40

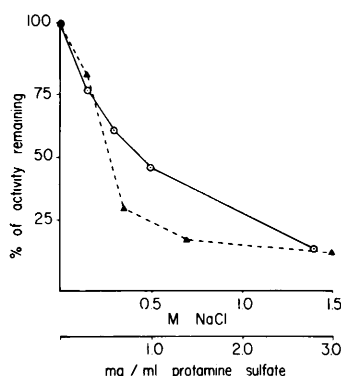

FIG. 5. Inhibition of aortic lipolytic activity by protamine sulfate (- -△- -) and sodium chloride (—⊙—). The assay conditions were those described in the Methods section.

greatly inhibited by NaF (20). At an optimum concentration for inhibition, 0.01 *M*, the bovine lipoprotein lipase showed no decrease in activity. A stimulation of the enzymatic activity was observed upon addition of heparin or Ca^{2+}. The Ca^{2+} effect is quite small, which is consistent with results obtained by Greten (21) on purified rat adipose lipoprotein lipase. The heparin stimulation was less than reported elsewhere (13); however, the aortic homogenate contains mucopolysaccharides which might substitute for heparin, causing a smaller apparent activation.

A crude sectioning of the artery was performed in order to determine the approximate location and concentration of lipoprotein lipase activity. The results of the experiment are shown in Table II. The highest specific activity section is that closest

TABLE II. LOCALIZATION OF LIPOPROTEIN LIPASE ACTIVITY IN THE BOVINE AORTA. (See text for details.)

	Expt. I		*Expt. II*	
	LPL spec. act. (units[a]/mg)	Total act. (units)	LPL spec. act. (units/mg)	Total act. (units)
Section I	20	190	20	190
Section II	11	630	7	270
Section III	5	504	3	216

[a] One unit equals one nmole FA released/hr. The activity values represent triolein hydrolysis that was inhibitable by protamine sulfate (1 mg/ml).

to the inner or lumen side of the artery. It consists of intimal cells scraped from the inner wall. The specific activity decreases toward the outer artery or adventitia. A high variability in the amount of lipoprotein lipase activity in aortas of different animals has been noted; however, the localization of the activity at the endothelial side of the artery has been consistently observed.

Conclusion. An identification of lipoprotein lipase in the bovine artery has been made. The specific activity of the enzyme is approximately 10% of that of rat adipose lipoprotein lipase in a comparable state (21), and 2% of that of post-heparin human serum (10). The measurement of such relatively low activity was made possible by the use of a sensitive assay system which incorporates the procedures of several other laboratories. The use of heated rat plasma as activator made less imperative the isolation of lipoprotein lipase activators such as apo C II which activate without adding lipolytic activity. A drawback is that lipase inhibitors endogenous to the plasma may remain.

The enzyme is found to have the highest specific activity in the intimal region of the artery, which lends support to Zilversmit's proposal (3) linking atherogenesis to lipoprotein lipase activity in the artery.

Summary. A lipoprotein lipase in the bovine arterial wall has been identified and partially characterized. The enzyme has a K_m apparent of 1 m*M* for triolein in a

phosphatidylcholine stabilized emulsion. The lipase was stimulated 20- to 30-fold by the addition of heated rat plasma to the assay medium. The activity exhibited a pH optimum at 8.6. Protamine sulfate (1.0 mg/ml) inhibited the activity by 50%, whereas 1.4 *M* sodium chloride inhibited by 85%. Sodium fluoride, an inhibitor of the hormone-sensitive lipase, had no effect on the activity. Additions of low concentrations of heparin or Ca^{2+} to the enzyme caused a slight stimulation of the lipolytic activity. A crude sectioning of the aorta revealed specific activity of lipoprotein lipase to be highest at the endothelial side of the artery.

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