Activation of lipoprotein lipase by lipoprotein fractions of human serum

DENNIS M. BIER and RICHARD J. HAVEL

Cardiovascular Research Institute, Department of Medicine and Department of Pediatrics, University of California San Francisco, San Francisco, California 94122

ABSTRACT Triglycerides in fat emulsions are hydrolyzed by lipoprotein lipase only when they are "activated" by serum lipoproteins. The contribution of different lipoprotein fractions to hydrolysis of triglycerides in soybean oil emulsion was assessed by determining the quantity of lipoprotein fraction required to give half-maximal hydrolysis. Most of the activator property of whole serum from normolipidemic, postabsorptive subjects was in high density lipoproteins. Low density lipoproteins and serum from which all lipoprotein classes were removed had little or no activity. Also, little activator was present in guinea pig serum or in very low density poor serum from an individual with lecithin:cholesterol acyltransferase deficiency, both of which are deficient in high density lipoproteins. Human very low density lipoproteins are potent activators and are much more active than predicted from their content of high density lipoprotein–protein. Per unit weight of protein, very low density lipoproteins had 13 times the activity of high density lipoproteins. These observations suggest that one or more of the major apoproteins of very low density lipoproteins, present as a minor constituent of high density lipoproteins, may be required for the activation process.

SUPPLEMENTARY KEY WORDS serum lipoproteins · lecithin:cholesterol acyltransferase deficiency · guinea pig serum

In 1955 Korn described the lipolytic activity in extracts of acetone powder from rat hearts as "lipoprotein lipase" when he demonstrated that hydrolysis of triglyceride emulsions occurred only when they had been exposed to serum lipoproteins (1). He showed that a complex, which he called "activated substrate," was formed by the interaction of lipoproteins and triglyceride. In his experiments, Korn also showed that HDL was responsible for most of the activating property of whole serum. LDL (d 1.019–1.063) was much less active but accounted for up to 16% of the activity present in native serum. Serum fractions from which all major lipoprotein classes had been removed (d > 1.21) were inactive. Scanu and Page (2) confirmed Korn's work with HDL but demonstrated no optical clearing in an agar–coconut oil system when activation was attempted with LDL. Scanu subsequently showed that apoprotein–phospholipid complexes derived from serum HDL could also activate artificial substrates (3, 4). We have investigated further the role of serum lipoprotein fractions in formation of the enzyme–substrate complex and have found that VLDL, whose triglycerides are a substrate for the enzyme, are also an extraordinarily potent activator of this process.

METHODS

Preparation of Lipoprotein Fractions from Human Serum

Blood serum was obtained from healthy men who had been fasted for at least 12 hr. Lipoprotein fractions were prepared by sequential flotation in a Beckman preparative ultracentrifuge at 114,500 *g* (average) and 5–12°C for 16–24 hr according to standard techniques (5). EDTA in a final concentration of 0.04% was added at all stages to minimize degradative changes. To prepare VLDL, 5 ml of whole serum was layered under 2 ml of 0.15 M aqueous sodium chloride (saline) in a 40.3 rotor tube. After centrifugation for 20 hr, the tube was sliced, and the supernatant lipoproteins of d < 1.006 were again layered under saline in a second 40.3 rotor tube. The fraction was centrifuged as before and recovered

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Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; FFA, free fatty acids.

quantitatively. To prepare LDL, whole serum was centrifuged at its own density for 20 hr. The resultant infranatant solution (d > 1.006) was raised to solvent density 1.025 with concentrated salt solution (5) and recentrifuged as before. The resultant infranatant fraction (d > 1.025) was adjusted to d 1.055 by the same method and centrifuged for 24 hr. After tube slicing, the supernatant layer containing lipoproteins of d 1.025–1.055 was layered under a salt solution of d 1.055 and recentrifuged as before. The LDL was then dialyzed against 0.04% EDTA in saline. Additional LDL was prepared by sequential flotation as above using 0.15 M sodium chloride in D_2O (d 1.111) to adjust the density. In some cases, the infranatant solution from the first centrifugation at d 1.055 was recovered quantitatively and saved for assay. To prepare serum free from VLDL and LDL, serum was raised to solvent density 1.063 or 1.085 with isotonic D_2O and centrifuged for 24 hr. The infranatant fraction (d > 1.063 or >1.085) was recovered quantitatively. A single purified HDL fraction (d 1.070–1.21) and a single pooled HDL subfraction of d 1.125–1.21 (HDL_3) were prepared by repetitive ultracentrifugation with concentrated salt solutions, recovered quantitatively, and dialyzed against 0.04% EDTA in saline. To prepare fractions free of major lipoproteins, infranatant fractions obtained after centrifugation at d 1.055 were made to solvent density 1.21 with solid KBr and centrifuged for 22 hr as described. The resultant infranatant fraction of d > 1.21 was layered under salt solution of d 1.21 and centrifuged as before. Salts were removed by dialysis against 0.04% EDTA in saline or by gel filtration on Sephadex G-25.

Guinea Pig Studies

Fresh guinea pig serum was obtained from male animals fasted for 48 hr. Postheparin plasma was obtained from animals fasted 48 hr and bled from the aorta under ether anesthesia 10 min after intravenous injection of 0.2 mg of heparin per kg of body weight. One part 0.1 M trisodium citrate was added to 10 parts of blood; the plasma was separated immediately by centrifugation at 2000 rpm and 5°C, and assayed within 2 hr.

Studies with Lecithin:Cholesterol Acyltransferase Deficient Serum

Two specimens (generously supplied by Dr. Kaare Norum, University of Oslo) from an individual (A.R.) with lecithin:cholesterol acyltransferase (LCAT) deficiency were tested as activator. Both specimens were refrigerated for 7–15 days prior to testing in the assay system. The sera were grossly lipemic, containing chylomicrons as well as VLDL.

Chylomicrons were removed from one specimen after centrifugation of the whole serum at 10^6 *g*-min. The infranatant solution was used as activator. Both VLDL and chylomicrons were removed from the second specimen of LCAT-deficient serum after layering the native serum under 0.15 M saline and centrifuging for 16 hr at 114,500 *g* and 12°C. The tubes were sliced, and the infranatant fraction (d > 1.006) was used as activator.

Preparation of Cows' Milk Proteins

Cows' milk is a rich source of lipoprotein lipase (6). Fresh milk was obtained from a local dairy. The raw milk was centrifuged at 5000 rpm and 5°C for 20 min. The supernatant cream layer was removed, and the infranatant layer was transferred to new tubes and recentrifuged for 30 min at 5000 rpm and 5°C. After dialysis, the suspension was lyophilyzed. The dried powder (stable for up to 1 yr when stored at −20°C) was dissolved in water to make a 4% solution.

Determination of Lipoprotein Lipase Activity

Lipoprotein lipase activity was determined by a modification of the method of Boberg and Carlson (7). The substrate mixture consisted of 0.024 ml of 10% soybean oil emulsion (Intralipid, Vitrum) per ml of ammonia–ammonium chloride buffer (11 volumes of 0.1 M NH_4OH plus 25 volumes of 0.1 M NH_4Cl containing 53 mg of human serum albumin and adjusted to pH 8.6). To prepare the incubation medium, 6 ml of the substrate mixture was added to an Erlenmeyer flask. Different volumes of serum or of lipoprotein fractions were incubated with the substrate for 30 min at 37°C before addition of enzyme. $^1/_2$ ml of enzyme solution was added, and the volume was made to 8 ml with saline. The final triglyceride concentration was 1.8 mg/ml of incubation medium, rather than 7.5 mg/ml as described by Boberg and Carlson (7). This concentration was chosen because it provided about 80% of maximal fatty acid release in our system and proportionately decreased the amount of lipoprotein needed to activate the substrate. The reaction was allowed to proceed at 37°C in a Dubnoff incubator shaker. After 15, 30, and 45 min incubation, 1 ml of the medium was extracted in duplicate into Dole's mixture (8). After separation of the phases, FFA was measured in a sample of the heptane layer by automated one-phase titration (9). Production of FFA was linear with all fractions tested. Enzyme activity was calculated as μmoles of FFA released per ml of enzyme solution per min. All values reported are corrected for hydrolysis in a control sample to which no lipoprotein was added. When VLDL was tested, an additional control sample containing no Intralipid was utilized to measure hydrolysis of VLDL triglycerides. This value was less than 10% of maximal hydrolysis in presence of Intralipid in all cases.

Determination of Activator Property

To compare the relative activities of the different lipoprotein classes, studies were performed with increasing amounts of whole serum or ultracentrifugal fractions thereof. Since the relation between quantity of activator and enzyme activity showed saturation kinetics, the quantity of activator required to give half-maximal reaction velocity (apparent K_m) was used to estimate its potency.

Chemical Methods

Lipids were measured by standard techniques (10, 11).

RESULTS

Normal Human Serum

Little or no hydrolysis of Intralipid triglycerides occurred during incubation in the absence of serum or lipoprotein fractions. A typical curve of lipoprotein lipase activity after preincubating the substrate mixture with increasing amounts of fresh whole serum is shown in Fig. 1. The apparent K_m with serum from normolipemic men was in the range of 0.12–0.16 ml or 0.015–0.020 ml per ml of incubation medium. Similar results were obtained with serum stored at 3°C for up to 4 wk. Two serum fractions from which all lipoprotein classes had been removed had almost no detectable activity (less than 0.025 μmoles of FFA per ml·min) up to volumes equivalent to 0.2 ml per ml of medium. Four serum fractions, two of d > 1.055 and two of d > 1.063, were prepared from the serum of a healthy man on four separate occasions using isotonic D_2O (Fig. 2). Addition of larger amounts of these fractions, in other experiments, produced little additional increase in enzyme activity. Since virtually no activator was found in serum fractions with d > 1.21, all of the activity in the d > 1.055 and 1.063 fractions can be attributed to HDL. From the apparent K_m of 0.024 ml of serum equivalent per ml of incubation medium, it appeared that HDL accounted for most, but not all, of the activity in serum. Activation by LDL was, however, very slight (Fig. 2). Fractions separated either with salt solutions or with isotonic D_2O gave similar results. Only 3–5% of the activity of whole serum could be attributed to LDL. VLDL was a potent activator and accounted for the remainder of the activity of whole serum. Sera from which VLDL had been removed, uniformly had appreciably lower content of activator. As shown in Table 1, removal of LDL had little further effect, confirming the limited activity of this lipoprotein fraction. In one experiment, additional removal of HDL_1 by centrifugation at d 1.085 had no additional effect. Since the protein content of VLDL in our subjects was less than 5% that of HDL, it appeared that VLDL might contain a higher concentration of activator per unit of protein. Table 2 demonstrates that for four subjects the average quantity of HDL protein required to produce half-maximal activation of substrate (46 μg/ml of medium) was 13 times that for VLDL protein (3.5 μg/ml of medium). For a single fraction of purified HDL (d 1.070–1.21) from subject 1, the equivalent amount of protein was 52 μg/ml of medium, and for a sample of HDL_3 (d 1.125–1.21) obtained from pooled sera of subjects 1, 2, and 3, the value was 60 μg/ml of medium. In these four subjects, VLDL accounted for 27–50% of the activator property of whole serum.

Abnormal Human Serum

Serum (free of VLDL and [or] chylomicrons as described) from an individual with LCAT deficiency was tested as activator because of the low concentration of HDL in serum of individuals with this inborn error of metabolism (14). No activity was detected in LCAT-deficient serum fraction from which chylomicrons and VLDL had been removed (<0.010 μmoles of FFA per ml·min with addition of increasing amounts from 0.15 to 3.3 ml of serum equivalent). With increasing amounts of serum from which chylomicrons alone had been re-

TABLE 1 CONTENT OF ACTIVATOR IN SERUM AND ULTRACENTRIFUGAL FRACTIONS FROM A NORMOLIPIDEMIC MAN

Experiment No.	Fraction	Volume of Fraction at apparent K_m
		ml of serum or equivalent per ml of medium
1	serum	0.021
	d>1.006	0.031
	d>1.063	0.034
2	d>1.006	0.030
	d>1.055	0.034
	d>1.085	0.034

TABLE 2 CONCENTRATION OF ACTIVATOR IN SERUM LIPOPROTEIN FRACTIONS

Subject	Concentration of Lipoprotein Protein at apparent K_m		Percentage of Whole Serum Activity in VLDL
	VLDL*	HDL†	
	μg of protein per ml of medium		
1	2.8	36	32
2	5.4	40	27
3	3.2	50	42
4	2.8	59	50
Mean	3.5	46	38
SEM	0.62	5.2	5.1

* Estimated from the triglyceride content with a normal ratio of triglycerides to protein of 5:1 (12).

† Estimated from the total cholesterol of d>1.055 fraction with ratio of total cholesterol to HDL protein of 1:4 (13).

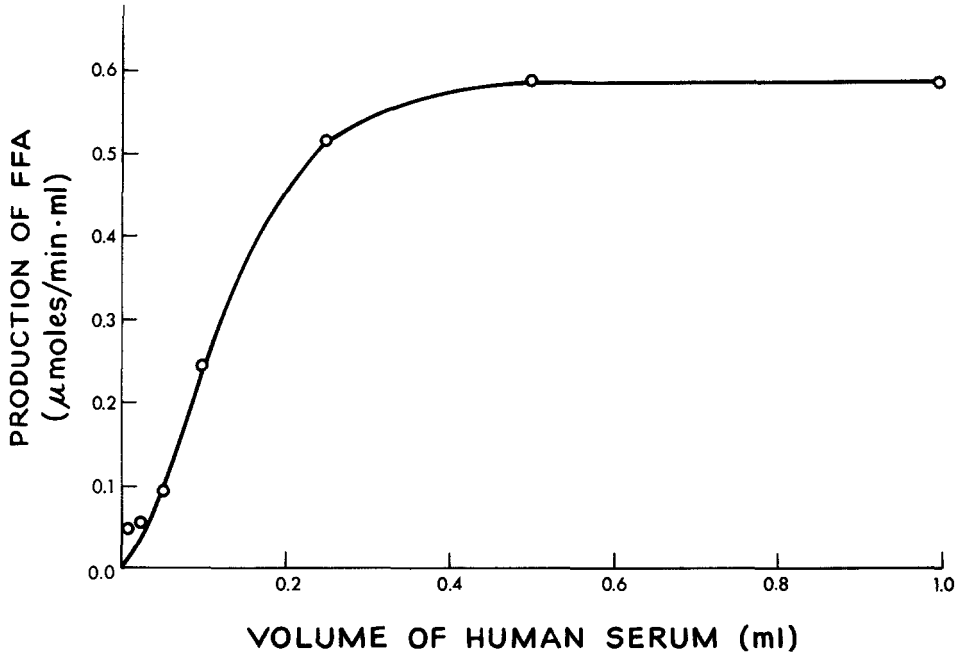

FIG. 1. Lipoprotein lipase activity of 1 ml of a 4% solution of milk proteins following preincubation of substrate mixture with increasing amount of fresh whole serum. Final volume of incubation mixture was 8 ml.

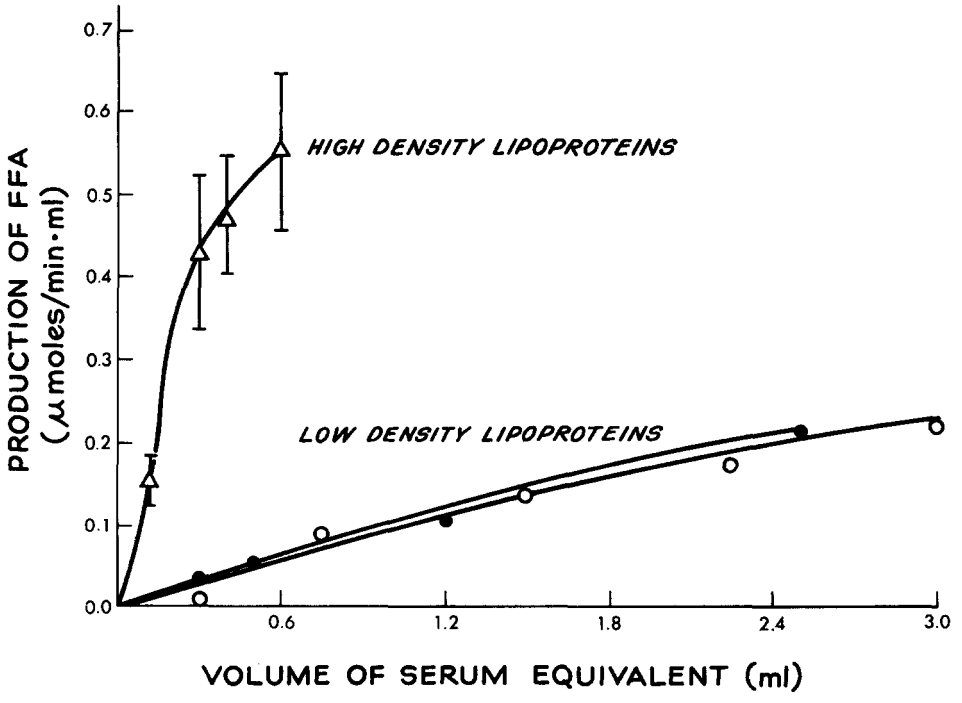

FIG. 2. Lipoprotein lipase activity following preincubation of substrate mixture with serum fractions containing HDL, d > 1.055 or 1.063, (△-△, mean of four experiments ± SEM) or with LDL, d 1.025–1.055, prepared with concentrated salt solutions (○-○) or with isotonic D_2O (●-●).

moved, activity initially increased normally, but maximal activity obtained after addition of 1 ml of serum (0.195 μmoles/ml·min) was low in comparison with a normal serum control (0.466 μmoles/ml·min).

Animal Studies

Because of the almost complete lack of HDL in the serum of adult guinea pigs (15, 16) and the observation by Whayne and Felts (17) that guinea pig postheparin

TABLE 3 ACTIVITY OF LIPOPROTEIN LIPASE FROM GUINEA PIG POSTHEPARIN PLASMA ON VARIOUS SUBSTRATES

Substrate	Triglyceride	Activity
	mg/ml of medium	*μmoles/min·ml*
Intralipid	7.5	0.07
"Activated" intralipid	7.5	0.22
Human chylomicrons	9.3	0.24

plasma lacked lipoprotein lipase activity, we evaluated the activator property of serum from guinea pigs. There was virtually no activation (<0.025 μmoles FFA/ml·min) with the addition of up to 1 ml of guinea pig serum from each of two adult male animals. Postheparin plasma obtained from three guinea pigs had little lipase activity when tested against Intralipid (7). Substantial activity was demonstrated when the artificial substrate was preincubated with human serum or if human chylomicrons were used as substrate. Table 3 shows the results of one such experiment.

DISCUSSION

The mechanism by which lipoprotein lipase acts on its natural substrates is unknown. The small amount of protein at the surface of chylomicrons and VLDL presumably permits formation of an enzyme–substrate complex essential for hydrolysis of the triglycerides contained in the central oil droplet. This hypothesis is based on the observations that triglycerides in artificial emulsions containing no lipoproteins, such as soybean oil emulsion used in the present studies, are not hydrolyzed by lipoprotein lipase in the absence of added lipoprotein (1) or apolipoprotein–phospholipid complex (3, 4). In the absence of lipoprotein, binding of enzyme to the emulsion and hydrolysis of contained triglycerides do not occur.

Our studies show that, as for enzyme from rat heart (1) and human postheparin plasma (2), the activator property for lipoprotein lipase in cows' milk is limited to lipoproteins. Whether the activator properties of different lipoprotein fractions observed in this study apply to lipoprotein lipase from other species is not known. However, HDL is a more potent activator than LDL for lipoprotein lipase from rat heart (1) and human postheparin plasma (2) as well as from cows' milk. In our studies, HDL contains most of the activator property in whole serum from postabsorptive subjects. Preparation of one of our low density fractions and of four fractions freed from VLDL and LDL with isotonic D_2O, eliminated the possible effects of high ionic strength on the lipoproteins and the known inhibitory effects of high salt concentrations on enzyme activity. These results are supported by our observations of the virtual absence of activator property in LCAT-deficient serum from which chylomicrons and VLDL had been removed and of the lack of activator in guinea pig serum. Our experiments with guinea pig postheparin plasma also explain the results of Whayne and Felts (17). In their experiments, lipoprotein lipase was undetectable in guinea pig postheparin plasma because the plasma lacked the lipoprotein activator needed to permit formation of an enzyme–substrate complex.

Of particular interest, however, is our observation that VLDL has much more of the activator property than would be expected from the amount of high density lipoprotein–protein normally present in the VLDL fraction (18, 19). Since apoprotein–phospholipid complexes derived from HDL can serve as activator (3, 4), one may postulate that specific apoproteins are the determinants of this property. When the activity of serum lipoprotein fractions was calculated per unit of protein, VLDL was 13 times as potent as HDL. At the apparent K_m for activation, the amount of VLDL protein in the incubation mixture (3.5 μg/ml of medium) was about 0.2% by weight that of the Intralipid triglycerides. Since Intralipid is emulsified with egg yolk lecithin in the amount of 12% of the weight of the triglycerides, it is unlikely that activation results from nonspecific changes in the surface of the emulsion particles. Rather it appears that a specific alteration of the surface is involved. This is further supported by the extremely low activity of LDL which has an apparent K_m for activation on the order of 500 μg of protein per ml of medium.

Recent studies have shown that the two major apolipoproteins of HDL, characterized by carboxyl-terminal threonine and glutamine (18), are minor constituents of VLDL (19). The major apolipoproteins of VLDL include that of LDL and certain additional polypeptides (18, 19). Only the latter are present in HDL and as minor constituents (18). From these considerations, it is attractive to postulate that one or more of these recently characterized polypeptides may participate specifically in the activation process.

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