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HUMAN LIPOPROTEIN LIPASE

II. INHIBITION OF ENZYME ACTIVITY BY PLASMA LOW DENSITY LIPOPROTEINS

C. J. FIELDING*
Department of Biochemistry, University of Oxford, Oxford (Great Britain)

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SUMMARY

1. When lipoprotein lipase is incubated with triglyceride in the presence of a mixture of plasma lipoproteins, the rate of enzyme activity is dependent upon the relative proportions of the lipoprotein fractions present.

2. The addition of low density or very low density lipoproteins to enzyme in such a mixture reduces the rate of enzymatic hydrolysis of triglycerides.

3. Enzyme activity migrates with the high density and low density lipoproteins during electrophoresis, apparently due to the formation of enzyme–lipoprotein complexes even in the absence of substrate triglyceride.

INTRODUCTION

Each of the lipoprotein fractions of human plasma can act as a co-factor for the hydrolysis of triglyceride emulsions by lipoprotein lipase[1]. Nevertheless, the rate of enzyme action is very dependent upon the identity of the lipoprotein. In whole plasma, a mixture of lipoproteins is present[2] and in the present paper the rate of lipoprotein lipase activity in the presence of mixtures of lipoproteins and of whole serum has been investigated. The results obtained suggest that the low density and very low density classes of lipoproteins are powerful competitive inhibitors of the enzymatic hydrolysis of triglyceride activated by high density lipoprotein or chylomicrons.

MATERIALS AND METHODS

Purified lipoprotein lipase, 2200–2250 units/mg protein, was obtained from the post-heparin plasma of human male donors as previously described[1]. This preparation retained no detectable activity against triglyceride in the absence of added lipoprotein. Pure lipoproteins were obtained from pooled male plasma[3]. Concen-

* Present address: Department of Medicine, University of Chicago, Chicago, Ill. U.S.A.

trations of lipoproteins have been expressed in mg/ml, calculated from the protein content of the fraction by use of the appropriate weighting factor to allow for the lipid content[2].

Electrophoresis of enzyme–lipoprotein complexes was carried out in slabs of 2.75% acrylamide gel[4]. Lipoprotein lipase was assayed using Intralipid 20% triglyceride emulsion (Vitrum, Sweden) activated with plasma as substrate[5], except where otherwise specified. Enzyme activity is expressed in terms of μmoles unesterified fatty acid released/h at 37° from this substrate.

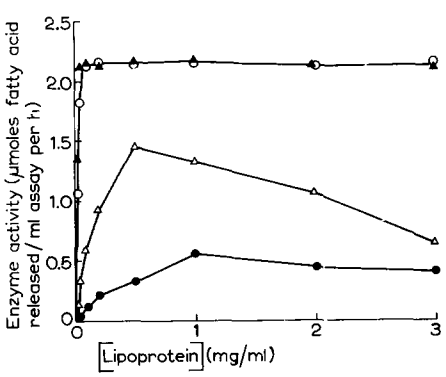
RESULTS

Lipoprotein requirement of lipoprotein lipase

The ability of chylomicrons, very low density, low density and high density lipoproteins to support the hydrolysis of triglyceride emulsion is shown as a function of lipoprotein concentration in Fig. 1. Although at very low concentrations chylomicrons were more effective than high density lipoprotein both fractions gave the same maximal activity at concentrations above 0.05 mg/ml. On the contrary, not only was the rate of hydrolysis in the presence of either low density or very low density lipoproteins always lower, but at concentrations above 1.0 mg/ml an inhibition of the maximal rate was found.

Inhibition of activity in mixtures of lipoproteins

In view of the lower rates of enzyme activity promoted by low density and very low density lipoproteins, experiments were carried out to determine whether these competed effectively with high density lipoprotein and chylomicrons in mixtures of lipoproteins. As shown in Fig. 2, addition of low density lipoprotein to high density lipoprotein in this system significantly inhibited the rate of fatty acid production by lipoprotein lipase. Inhibition was also obtained by the addition of very low density



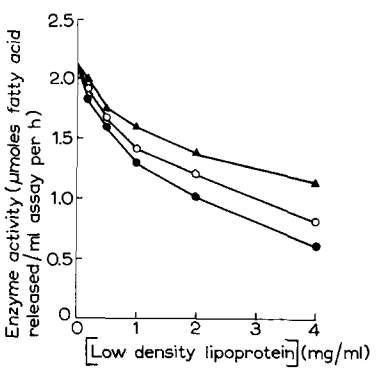


Fig. 1. Activation of lipoprotein lipase by a single class of lipoproteins. Assays were carried out using lipopreotein fractions in place of whole serum. Initial total triglyceride was, in each case, 2.0 μmoles/ml. Incubation for 1 h at 37°. ▲———▲, chylomicrons; △———△, very low density lipoproteins; ●———●, low density lipoproteins; ○———○, high density lipoprotein.

Fig. 2. Competition between low density lipoprotein and high density lipoprotein as co-factors of lipoprotein lipase. High density lipoprotein concentrations: ●———●, 1.0 mg/ml; ○———○, 2.7 mg/ml; ▲———▲, 4.2 mg/ml. Assay conditions were as shown in Fig. 1.

lipoprotein to high density lipoprotein under the same conditions. The inhibition by very low density lipoprotein of the activity promoted by high density lipoprotein concentrations of 1.0, 2.7 and 4.2 mg/ml was found to be between 30 and 40% of that given under the same conditions by low density lipoprotein. Both very low density and low density lipoproteins also inhibited the activity promoted by chylomicrons. Inhibition by low density lipoprotein was 30%, and by very low density lipoprotein 20%, of that inhibition given by low density lipoprotein of high density lipoprotein-mediated activity (Fig. 1), over the range of chylomicron concentrations up to 1.0 mg/ml.

Centrifugation of lipoprotein lipase in the presence of lipoproteins

When post-heparin plasma containing lipoprotein lipase is incubated with triglyceride a stable enzyme–lipoprotein triglyceride complex is formed that rises to the meniscus as a fatty layer after centrifugation[6,7]. To further investigate the interaction of the enzyme with lipoproteins in the presence of substrate triglyceride, the flotation behaviour during centrifugation of lipoprotein lipase in the presence of triglyceride and isolated lipoproteins was examined. Enzyme in the presence of chylomicrons or high density lipoprotein was almost completely recovered in the super-

TABLE I

RECOVERY OF LIPOPROTEIN LIPASE AFTER CENTRIFUGATION

0.30 ml of enzyme solution (50–60 units/ml) was mixed with the same volume of lipoprotein solution (4.0 mg/ml) or whole plasma in 0.145 M NaCl, and with triglyceride emulsion to a final concentration of 5 μmoles/ml triglyceride. The mixture was incubated for 20 min at 37°, cooled, then 0.5 ml was layered under 4.5 ml of 0.05 M NH_4OH–NH_4Cl buffer (pH 8.3) and centrifuged at 0° for 30 min at 20 000 rev./min. in the SW-39 rotor of the Spinco Model L ultracentrifuge. The tubes were sliced 1 cm below the meniscus and the top and bottom fractions assayed. Recoveries are expressed as the total enzyme units in the fraction.

Fraction	*Chylomicrons*	*Lipoprotein co-factor*			*Whole plasma*
		Very low density lipoprotein	*Low density lipoprotein*	*High density lipoprotein*	
Supernatant	17.0	8.9	3.2	16.3	4.9
Infranatant	0.8	9.0	14.8	1.8	11.8

natant lipid layer (Table I). Much less enzyme–very low density lipoprotein or enzyme–low density lipoprotein complex was found in the supernatant under the same conditions.

Effects of whole plasma upon lipoprotein lipase activity

Experiments were carried out to determine the rate of enzymatic hydrolysis in the presence of whole human plasma as compared with that found with isolated lipoproteins.

Blood was taken from healthy male donors, 20–30 years, on normal diet. 5-ml samples were taken from a brachial vein into 1/40 vol. of 0.25 M sodium citrate. After centrifugation at 20 000 × *g* for 30 min to remove both red cells and platelets, the supernatant plasma was incorporated into 5-ml samples of assay medium[5] (0.2 ml

plasma/ml assay medium). At the same time media were made up to contain solutions of either high density lipoprotein only (2.0 mg/ml) or a mixture of purified lipoproteins in concentrations approximating to those present in normal male plasma[2] (very low density lipoprotein, 1.6 mg/ml; low density lipoprotein, 3.7 mg/ml; high density lipoprotein, 2.8 mg/ml) diluted as for plasma. After addition of 0.1 ml purified enzyme to each assay, duplicate 1-ml samples were taken for analysis of unesterified fatty acid content before and after incubation for 1 h at 37°.

The amount of enzyme activity in the samples containing plasma was approximately half that found when only high density lipoprotein was present (high density lipoprotein samples 16.0 units/ml enzyme solution; plasma samples, 8.5 ± 1.4 units (range 6.2–10.3 units, fifteen samples)). In assays of enzyme in the presence of the mixture of lipoproteins, the activity was 8.1 units/ml enzyme solution. This result suggested that the inhibitory effect of whole plasma was accounted for by the very low density lipoprotein and low density lipoprotein present.

Electrophoresis of enzyme–lipoprotein complexes

Only chylomicrons, and to a lesser extent very low density lipoprotein, serve as enzyme substrates in the absence of added triglyceride[8]. It was of interest to determine whether low density or high density lipoproteins would form complexes with lipoprotein lipase when substrate was not present.

Electrophoresis gels were prepared from cyanogum 41 (2.75%), *N,N,N',N'*-tetramethylethylenediamine (0.15%) in Tris (25 mM)–glycine (190 mM) buffer (pH 8.3) and were polymerized with 0.20% ammonium persulphate. The same buffer was contained in the electrode vessels. The slab was cooled with circulating ice water during the experiments.

After passage of a current of 5 mA (100 V) for 1 h to remove possible oxidizing contaminants from the polymerization process, 60 μl of sample was applied to each slot of the gel. Samples contained 30 μl of purified lipoprotein lipase, 100–200 units/ml, 15 μl of 6% (w/v) bovine serum albumin in Krebs–Ringer solution made 0.25 M with respect to Tris–HCl buffer (pH 8.3) and 0.15 μl of either whole pooled human plasma or isolated lipoprotein fraction (6 mg/ml) in 0.145 M NaCl. The mixtures were incubated for 5 min at 37° before addition to the electrophoresis gel. The initial current was 5 mA and did not vary significantly during the experiment. Electrophoresis was carried out for 12–14 h.

The gels were then divided lengthwise, and 2-mm sections of one-half were homogenized in 2.5 ml of assay medium using a ground glass homogenizer. Assays were carried out for 2 h at 37°, and duplicate 1-ml samples taken for measurement of enzyme activity. The other half was sectioned similarly, and the triglyceride content of the gel fraction was determined as glycerol[8] after hydrolysis with alcoholic KOH.

In the presence of high density liprotein the enzyme migrated with this lipoprotein (Fig. 3). In the same way when low density lipoprotein was the lipoprotein present, enzyme activity was associated during migration with this lipoprotein. Enzyme preincubated with plasma gave two peaks of activity after electrophoresis which corresponded to those obtained with high density lipoprotein and low density lipoprotein alone. No enzyme activity was recovered when electrophoresis was carried out in the absence of lipoprotein.

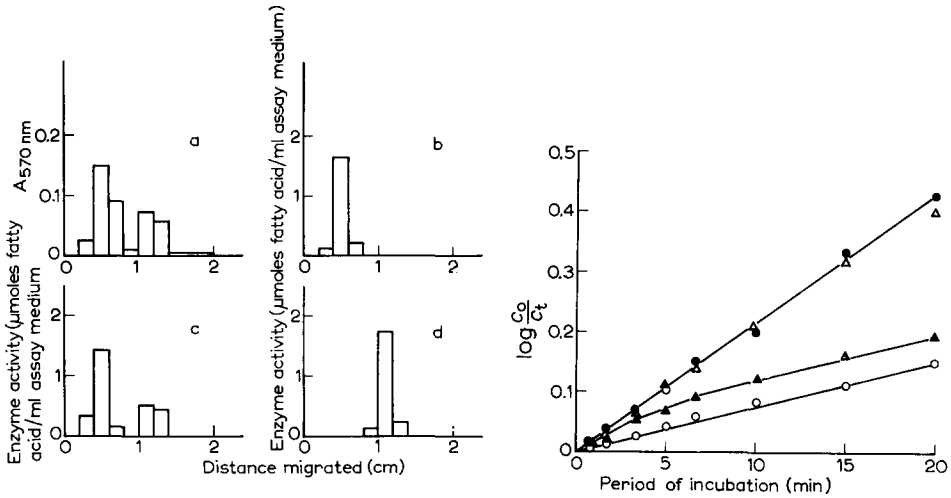


Fig. 3. Electrophoresis of enzyme–lipoprotein complexes. The experimental conditions are described in the text. a. Neutral glyceride content of plasma after electrophoresis. b. Enzyme activity after electrophoresis from enzyme + low density lipoprotein. c. Activity from enzyme + plasma. d. Activity from enzyme + high density lipoprotein.

Fig. 4. Stability of lipoprotein lipase to incubation at 37°. The proportion of enzyme activity remaining after a given period of incubation is expressed as the log of the initial concentration in enzyme units (c_0) to concentration (c_x) after an incubation period of t_x min. ●———●, enzyme; ○———○, enzyme + high density lipoprotein; △———△, enzyme + low density lipoprotein; ▲———▲, enzyme + plasma.

Stabilization of enzyme activity by lipoproteins

The previous experiment suggested the formation of enzyme–high density lipoprotein and enzyme–low density lipoprotein complexes even under conditions where no substrate triglyceride was present. The stability of the enzyme under such conditions was investigated.

Purified enzyme solution (10–20 units/ml) was mixed with 0.5 vol. of albumin–Tris buffer and 0.5 vol. of plasma or lipoprotein solution as described in the previous experiments. Mixtures were incubated at 37°, and duplicate samples were taken at zero time and at intervals during the incubation, and added to normal assay medium, to determine the rate of loss of activity from the enzyme in the presence and absence of lipoproteins (Fig. 4).

In the absence of lipoproteins, or in the presence of low density lipoprotein only, activity was rapidly lost according to first-order kinetics. Significant stabilization was given in the presence of high density lipoprotein or of whole plasma. The rate of loss of activity from whole plasma suggests the presence of a mixture of stable and unstable elements; an initial rapid loss of activity was followed by a slower rate of decline later in the incubation (Fig. 4).

DISCUSSION

The results obtained suggest that when lipoprotein lipase acts upon triglyceride in the presence of a mixture of lipoproteins such as that found in whole plasma, the

rate of enzyme activity is significantly dependent upon the absolute concentrations and relative proportions of the lipoproteins fractions present.

The concentration dependence of the effectiveness of lipoproteins as co-factors of the hydrolysis of triglyceride is shown in Fig. 1. A very low concentration of chylomicrons or high density lipoprotein is sufficient to provide a maximal rate of activity. However, not only is the rate with low density lipoprotein and very low density lipoproteins always lower, but at concentrations above 1.0 mg/ml a further decrease in activity occurs. This inhibition remains unexplained, although the similarity of the concentration curves for both co-factors appears to be evidence of their functional similarity. A significant inhibition of high density lipoprotein-mediated activity is obtained by the addition of increasing amounts of low density lipoprotein (Fig. 2), and also for the inhibition of high density lipoprotein by very low density lipoprotein and of chylomicrons by very low density lipoprotein and low density lipoprotein. The concentration ranges investigated included those typically found in whole human plasma[2] and a significantly lower enzyme activity is found when plasma is contained in the assay medium than when pure high density lipoprotein is used. The similarity of the activities obtained from artificial mixtures of lipoproteins and from whole plasma suggests that the principal inhibitory effect is provided by the very low density lipoprotein and especially the low density lipoprotein of the plasma. Inhibition of the enzyme by an unidentified plasma factor has been previously reported[9,10] and it is possible that this factor can be identified with the low density and very low density lipoprotein content of the plasma.

The separation of enzyme activities associated with the low density and high density lipoprotein bands during electrophoresis, together with the marked stabilization of enzyme in the presence of high density lipoprotein, provides evidence of the existence of enzyme–low density lipoprotein and enzyme–high density lipoprotein complexes, even in the absence of substrate. The previous demonstration of two bands of enzyme activity after the electrophoresis of whole post-heparin plasma, in the α- and β-globulin bands[9], suggests that under these conditions also such enzyme–lipoprotein complexes are present. Such complexes in the presence of excess triglyceride could also be separated by their flotation behaviour in the ultracentrifuge (Table I).

The role of lipoproteins in the activity of lipoprotein lipase appears to involve significant effects upon both the rate of hydrolysis of triglyceride, and also the stability of the activity in whole plasma. It is not clear whether the difference in the properties of high density and low density lipoproteins in this system involves the lipid or protein moieties, or both. The system described should be suitable for an investigation of the detailed roles of these components in the activity of lipoprotein lipase.

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