

## A rapid assay for lipoprotein lipase

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**SUMMARY** A rapid assay for lipoprotein lipase activity employing a  $^{14}\text{C}$ -labeled substrate is described. The method is very sensitive and suitable for routine use.

**SUPPLEMENTARY KEY WORDS** triolein · lipase

LIPOPROTEIN LIPASE is an enzyme which is thought to play a regulatory role in fat deposition. Although this enzyme has been extensively studied for a number of years, a recurring problem has been the choice of a substrate for measuring its activity. Usual choices have been naturally occurring chylomicrons (1) or commercially available fat emulsions (2) such as Ediol (Lipostat; Calbiochem, Los Angeles, Calif.), Intralipid (AB Vitrum, Stockholm, Sweden), and Lipomul (The Upjohn Co., Kalamazoo, Mich.). The use of chylomicrons as a substrate for routine assay offers a number of difficulties with regard to preparation, availability, standardization, and storage. Thus many investigators have preferred to use commercial fat emulsions in their assay systems. These preparations are generally composed of mixed glycerides. For instance, Ediol, a coconut oil emulsion commonly employed in lipase assays, contains approximately 4% di- and monoglycerides (3). Since more than one lipase is usually present in crude extracts from adipose tissue it is difficult, if not impossible, to determine what part of the enzyme activity measured by the appearance of fatty acid is derived from the mono-, di-, or triglyceride contained in the Ediol. Recently, Greden, Levy, and Fredrickson (4) have described an assay for lipoprotein lipase activity in which a pure radioactive triglyceride is used. We also have employed a pure radioactive triglyceride in order to retain specificity. In addition, we have devised a rapid method for isolating and assaying the labeled fatty acids produced by the enzymatic hydrolysis.

**Materials.** Triolein- $^{12}\text{C}$  (purity >99%) was purchased from the Hormel Institute of the University of Minnesota, Austin, Minn., and the triolein- $^{14}\text{C}$  from Applied Science Laboratories Inc., State College, Pa. (84 mc/

mmole). (The purity of the radioactive triglyceride was checked by determining the radioactivity in the various fractions after thin-layer chromatography [5]. The non-radioactive triolein was chromatographed in a similar manner, and the lipid spots were made visible by iodine and Rhodamine G staining.)

Radioactivity was assayed with a scintillation mixture, Instagel, containing a nonionic surfactant (Packard Instrument Co., Inc., Downers Grove, Ill.). Triton X-100 (Rohm & Haas, Philadelphia, Pa.), and bovine albumin, Fraction V (Armour Pharmaceutical Co., Chicago, Ill.) were also employed in these experiments. The enzyme preparation used was a 0.025 M  $\text{NH}_4\text{OH}$  extract of rat adipose tissue obtained from rats fed ad lib. (2).

**Procedure.** Blood obtained from a dog which was fasted overnight was allowed to clot for 1 hr at  $23^\circ\text{C}$ . Serum was separated by centrifugation. A mixture of 1.0  $\mu\text{C}$  of triolein- $^{14}\text{C}$  and 0.133 g of triolein- $^{12}\text{C}$  was dried under a stream of nitrogen. 3 ml of serum, 0.9 ml of 1% bovine serum albumin (adjusted to pH 8.0), 0.9 ml of 1% Triton X-100, and 7.2 ml of 0.2 M Tris-HCl buffer (pH 8.0) were added to the triolein. The mixture was placed in an ice bath and was sonicated with a Biosonik II microprobe (Bronwill Scientific Div., Will Scientific, Inc., Rochester, N.Y.) for 4 min at maximum power output (125 w). The resultant mixture was sufficient for the assay of 15 samples. If more than 15 samples were to be assayed, multiple batches of the assay mixture were prepared. For a single assay 0.2 ml of enzyme extract was added to 0.8 ml of the above mixture and incubated for 20 min at  $37^\circ\text{C}$ . The reaction was terminated by addition of 4 ml of isopropanol-3 N  $\text{H}_2\text{SO}_4$  40:1. For the extraction of the lipids, 2 ml of  $\text{H}_2\text{O}$  and 5 ml of hexane were added (6), and the tube was shaken end-to-end on a mechanical shaker for 1 min. 20 min later a 5 ml aliquot of the hexane phase was added to 1 ml 0.1 N KOH. On shaking this latter mixture for 10 min, the free fatty acids were extracted into the alkali. The volume of the lower phase was recorded since this phase increased in volume after shaking. 1 ml of the KOH phase was dissolved slowly with 5 ml of Instagel, and the radioactivity was assayed in a Packard Liquid Scintillation Counter.

**Results.** The procedure used to remove fatty acids from the reaction mixture into hexane has been shown to extract completely long-chain fatty acids (6). We have tested the further extraction of long-chain fatty acids from this hexane phase into 0.1 N KOH as described above. When corrected for recorded volume changes due to phase mixing, this second extraction was found to be quantitative also. Furthermore, the per cent contamination of the alkali phase with radioactive triglyceride substrate was negligible, i.e., less than 0.1%.

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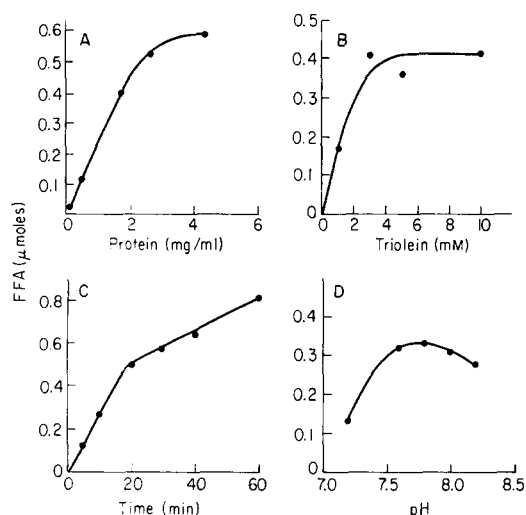


FIG. 1. Relation of lipoprotein lipase activity to (A) enzyme concentration, (B) substrate concentration, (C) incubation time, and (D) pH of assay. Conditions of the assay are described in the text. Results are expressed as  $\mu$ moles of free fatty acid produced per 20 min per ml of incubation medium.

Using this assay system, enzyme activity was linear with increasing enzyme concentration between 0.1 and 3.0 mg of protein (Fig. 1 A). The triolein substrate concentration was not rate limiting at 10 mM (Fig. 1 B). Enzyme activity was linear for the first 20 min of incubation (Fig. 1 C). Thereafter the slope changed, and, therefore, all incubations were terminated at 20 min. The activity was optimal at pH 7.8; however, the maximum was fairly broad (Fig. 1 D). In addition, optimal concentrations of dog serum and albumin were determined experimentally in the presence of a constant amount of enzyme so that further additions had no effect on the lipase activity.

The substrate mixture is stable for as long as 4 days at 4°C. Incubation of the substrate mixture at 37°C for periods up to 1 hr prior to addition of the enzyme does not alter the properties of the substrate. When the same enzyme preparation is assayed repeatedly the standard deviation is  $\pm 3\%$ .

Characteristics of the lipoprotein lipase assay system are shown in Table 1. The absence of serum or addition

TABLE 1 CHARACTERIZATION OF LIPOPROTEIN LIPASE ASSAY SYSTEM

Condition of Assay	Activity
Complete system	100
- Serum	13
Prior incubation with NaCl (1 M)	0
+ Heparin 1 $\mu$ g	100
+ Heparin 20 $\mu$ g	38

of 1 M NaCl reduces the level of activity to 10% of the control value. Addition of small amounts of heparin had no effect on the lipase activity, whereas 20  $\mu$ g additions resulted in a 60% inhibition.

The triglyceride substrate, triolein, which contains the same fatty acids in all ester bond positions may not be hydrolyzed at the same rate as a mixed triglyceride. Furthermore, the order in which the enzyme hydrolyzes the ester bond at the  $\alpha$  and  $\beta$  positions of the triolein cannot be ascertained. However, the assay is sensitive, specific, and extremely rapid, and should be useful for determining lipoprotein lipase activity during its purification or in routine metabolic studies.

*Addendum.* If the acetone powder of adipose tissue is extracted with cold 0.05 M  $\text{NH}_4\text{OH-NH}_4\text{Cl}$  buffer, pH 8.5, the lipolytic activity obtained is linear with respect to time for periods up to 60 min.

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