JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012

A simple lipase assay using trichloroacetic acid

Michael C. Schotz and Arlene S. Garfinkel

Research, Veterans Administration, Wadsworth Hospital Center, Los Angeles, California 90073, and Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, California 90024

Summary An extremely rapid and sensitive assay for lipoprotein lipase activity, suitable for routine determinations, is described. The substrate for the assay is emulsified [2-^{3}H]glyceryl trioleate, activated by serum. The method is based on trichloroacetic acid precipitation of unreacted substrate and measurement of ^{3}H-labeled glycerol.

Supplementary key words adipose tissue · postheparin plasma · triolein · ^{3}H-labeled glycerol · lipoprotein lipase · triglyceride

There have been reported a number of new lipase assays in which a single purified triglyceride was used for substrate rather than the mixture of glycerides contained in commercial emulsions (1–5). To increase speed and sensitivity of these assays, radioactive tracers have been incorporated in the triglyceride. In general, these methods rely on complicated procedures to separate radioactive products from substrates. The present report is concerned with an assay for lipoprotein lipase in which trichloroacetic acid is used to separate in one step radioactive glycerol hydrolyzed by the enzyme from residual ^{3}H-labeled glyceryl trioleate.

Materials. Sources of materials were as follows: glyceryl trioleate (purity > 99%), the Hormel Institute, University of Minnesota, Austin, Minn.; [2-^{3}H]glyceryl trioleate (266 mCi/mmole), Amersham/Searle, Arlington Heights, Ill.; bovine albumin, fatty acid-poor, Miles Research Products, Kankakee, Ill.; Triton X-100, Rohm and Haas, Philadelphia, Pa.; and egg lecithin, Schwarz/Mann, Orangeburg, N.Y. The egg lecithin was purified by a water wash of a chloroform–methanol 2:1 (v/v) extract (6). All other chemicals used were reagent grade.

Pasteur pipettes, 14.5 cm long, were plugged at the constriction with a small amount of glass wool (approximately 40 mg). The assay was carried out in a disposable glass test tube, 1.3 × 10 cm.

Enzyme sources were rat postheparin plasma (7) and NH_4OH–NH_4Cl buffer extracts of acetone powders of rat epididymal adipose tissue (8).

Procedure. Substrate was prepared by sonicating a mixture of 270 mg of glyceryl trioleate (containing 20 μCi of [2-^{3}H]glyceryl trioleate), 16.2 mg of egg lecithin, 3.0 ml of 10% fatty acid-poor albumin, adjusted to pH 8.0, 1.6 ml of 2 M Tris–HCl buffer, pH 8.2, and 1.4 ml of water. Sonication of this mixture was carried out in a plastic test tube, 1.8 × 10 cm, immersed in an ice bath. The microprobe of a Branson Sonifier (model LS 75, Branson Instruments, Stamford, Conn.) maximally tuned at the no. 2 setting was used. The substrate was sonicated for a total of 3 min, alternating a 1-min sonication with a 1-min pause. When larger quantities of this emulsion were required, several separate batches of the

Abbreviations: TCA, trichloroacetic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2(5-phenyloxazolyl)benzene.

6-ml mixture were prepared. This triglyceride emulsion was activated by incubation at 37°C for 30 min with 12 ml of serum obtained from dogs fasted overnight. A 0.6-ml aliquot of the activated substrate was then incubated at 37°C with enzyme preparations, in a total volume of 1 ml. Routinely, samples were incubated for 60 min; however, depending on the lipase preparation, shorter incubation times could be used. The reaction was terminated by addition of 1 ml of 10% (w/v) TCA to the 1-ml assay mixture. After brief mixing, the precipitate was sedimented by centrifugation for 5 min at 1000 *g*.

1 ml of the TCA supernate containing the newly formed glycerol was filtered through a glass wool-plugged Pasteur pipette (Fig. 1) into a counting vial, which contained 0.05 ml of 5 N NaOH. Delivery of the last drops from the Pasteur pipette into the counting vial was accomplished by blowing a stream of nitrogen gas into the pipette for approximately 5 sec.

To the aqueous TCA supernatant solution was added 8 ml of a mixture of 3 ml of Triton X-100 and 5 ml of 1% PPO in toluene; then 5 ml of liquid scintillator, consisting of 0.4% PPO and 0.01% POPOP in toluene, was added. In one experiment we compared the measurement of glycerol by this radioactive procedure with chemical analysis of glycerol (9).

The procedure is facilitated greatly by using mechanical pipetting devices to deliver all reagents and by sampling the TCA supernatant fraction with the Biopette (Schwarz/Mann).

Results. The method described above is based on the principle that triglyceride in the presence of protein is quantitatively precipitated by TCA, whereas free glycerol is soluble in 5% TCA. Any glycerol formed as a result of enzyme action is then readily separable from the residual substrate simply by separation of the precipitate from the supernatant fraction.

When serum is present in the assay mixture, the triglyceride presumably forms a complex with the serum proteins and is completely precipitated on addition of TCA (Table 1). In the absence of serum, interestingly, triglyceride is still completely precipitated by TCA addition (Table 1), apparently in association with albumin. The low number of counts present in the TCA supernatant fraction under these conditions (Table 1) was found consistently with substrates prepared and used on the same day. When substrates were stored at 4°C for 24–48 hr, the blank value doubled or tripled. Therefore, we routinely used substrates within 24 hr after preparation. In an experiment carried out with Dr. P. Belfrage in which tracer amounts of ^{3}H-labeled monoglyceride were added, 99% of the monoglyceride was precipitated by TCA. On the other hand, when tracer amounts of ^{3}H-labeled glycerol were added to the assay mixture, 100% of the glycerol was found in the TCA supernatant fraction.

TABLE 1. Precipitation of emulsified [2-^{3}H]glyceryl trioleate by 10% TCA

Fraction	Total cpm	
	With Serum	No Serum
TCA supernate	87	91
TCA precipitate	7×10^5	7×10^5

When the TCA supernatant fraction containing ^{3}H-labeled glycerol was assayed for ^{3}H, it was noted that these samples were quenched considerably. Addition of NaOH to the TCA supernate overcame the effect completely.

Using adipose tissue extracts, we found that the formation of glycerol was proportional to enzyme concentration up to 0.2 ml of adipose enzyme extract (4 mg of protein/ml) incubated for 1 hr (Fig. 2*A*). Assuming that 3 moles of fatty acid were produced per mole of glycerol produced, at least 2.1 μmoles of fatty acid were present in the assay vessel at the point of divergence from linearity

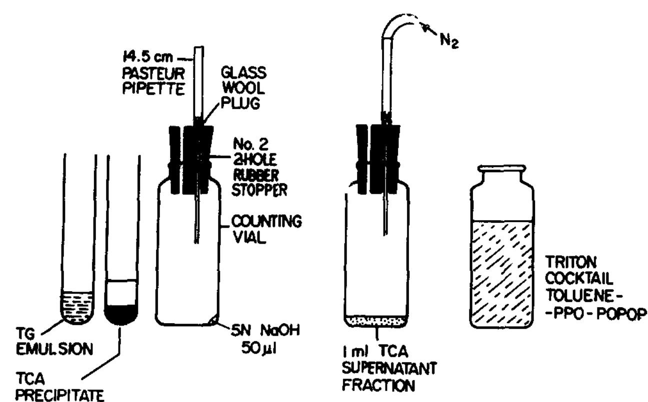

FIG. 1. Diagram of the procedure.

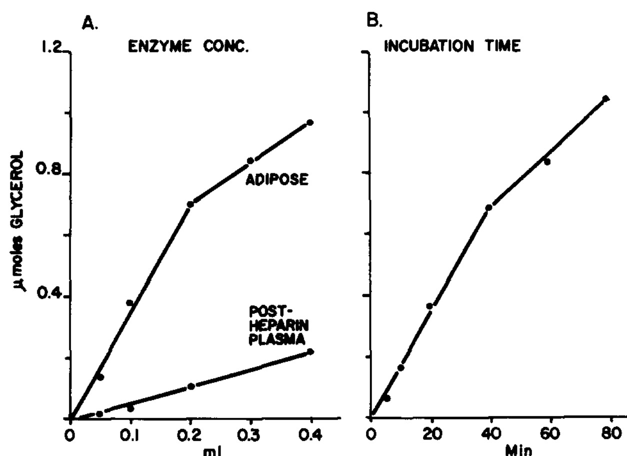

FIG. 2. Lipoprotein lipase activity relative to (*A*) enzyme concentration and (*B*) incubation time.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 2 Characterization of lipoprotein lipase assay system

Condition of Assay	Activity: Adipose Extract	Activity: Postheparin Plasma
	%	
Complete system	100	100
Minus serum	8	71
Prior incubation with NaCl (1 M)	6	11

TABLE 3. Comparison of radioactive and chemical measurements of glycerol produced in lipoprotein lipase assay

Sample No.	Glycerol: Radioactive	Glycerol: Chemical
	μmoles	
1	0.08	0.09
2	0.15	0.14
3	0.23	0.22
4	0.34	0.28

Different amounts of acetone powder extracts of adipose tissue were incubated for 60 min with triglyceride substrate as described in the text. Aliquots of the TCA supernatant solution were assayed for glycerol content by a chemical procedure (9) and by the radioactive procedure described in the text. The values reported are the means of duplicate determinations and represent μmoles of glycerol/ml of TCA supernatant solution.

of the curve of adipose enzyme concentration. A corresponding point diverging from linearity was observed when 0.3 ml of adipose enzyme extract was incubated for longer than 40 min (Fig. 2*B*). When postheparin plasma, diluted 10-fold, was employed as enzyme source, a linear relationship was observed up to 0.4 ml of diluted plasma incubated for 1 hr (Fig. 2*A*).

Lipoprotein lipase activity is markedly activated by serum and inhibited by 1 M NaCl (8). These properties were demonstrated in both adipose tissue extracts and postheparin plasma using the present method (Table 2). Dog serum was used for activation of the triglyceride substrate because it was easily available. Presumably, sera from other species would also activate the triglyceride substrate.

Comparison of the glycerol content of the TCA supernatant fraction determined by the radioactive assay and by chemical analysis showed good agreement (Table 3).

Since this method is dependent on only one critical step to separate the product from the substrate, it is highly reproducible. The procedure has proved to be invaluable in handling large numbers of samples for assay of lipoprotein lipase. All the glassware used is disposable; therefore, the method eliminates the danger of radioactive contamination and results in a large saving of time with regard to washing glassware. In addition, this method has the advantage that it does not require organic solvent extraction of the products.

This procedure should be applicable to other lipase assays. Because this method can detect as little as 1–2 nmoles of glycerol, the technique could be used for assay of lipoprotein lipase with 5 μl or less of postheparin plasma.

This work was supported in part by Public Health Service grant no. 4706 from the National Institute of Arthritis and Metabolic Diseases. The authors are very grateful to Richard Berquist for his assistance.

Manuscript received 27 July 1972; accepted 19 August 1972.

REFERENCES

1. Greten, H., R. I. Levy, and D. S. Frederickson. 1968. A further characterization of lipoprotein lipase. *Biochim. Biophys. Acta.* **164:** 185–194.
2. Belfrage, P., and M. Vaughan. 1969. Simple liquid–liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
3. Schotz, M. C., A. S. Garfinkel, R. J. Huebotter, and J. E. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* **11:** 68–69.
4. Kaplan, A. 1970. A simple radioactive assay for triglyceride lipase. *Anal. Biochem.* **33:** 218–225.
5. Posner, I., and V. Bosch. 1971. A method for the determination of lipoprotein lipase in postheparin plasma and body tissues utilizing a triolein-coated Celite substrate. *J. Lipid Res.* **12:** 768–772.
6. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
7. Schotz, M. C., and A. S. Garfinkel. 1972. Effect of nutrition on species of lipoprotein lipase. *Biochim. Biophys. Acta.* **270:** 472–478.
8. Garfinkel, A. S., and M. C. Schotz. 1972. Separation of molecular species of lipoprotein lipase from adipose tissue. *J. Lipid Res.* **13:** 63–68.
9. Korn, E. D. 1955. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. *J. Biol. Chem.* **215:** 1–14.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR