Hydrolysis of glyceryl tri[1-^{14}C]octanoate and glyceryl tri[1-^{14}C]oleate monolayers by postheparin lipolytic activity

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Abstract The hydrolysis of monomolecular films of glyceryl tri[1-^{14}C]octanoate and glyceryl tri[1-^{14}C]oleate has been demonstrated by measurement of the decrease in surface radioactivity that occurs in the presence of postheparin plasma. The hydrolysis displayed first order kinetics and was proportional to enzyme concentration over a 10-fold range. No hydrolysis was observed in the absence of enzyme, and only slight activity (1%) was found in plasma taken from subjects before heparin administration. The hydrolysis was stimulated to a variable extent by Ca^{2+}. The first product of hydrolysis of the monolayer was identified as 1,2-diglyceride, which was subsequently converted to 2-monoglyceride. Inhibition of triglyceride hydrolysis was observed when postheparin plasma was preincubated in 2 M NaCl, 10^{-4} M protamine, 10 mM $Na_4P_2O_7$, and 0.1 M NaF. Monolayer techniques avoid some but not all of the problems associated with emulsified lipid substrates and appear to be applicable for study of postheparin lipolytic activities.

Supplementary key words lipid monolayers · triglyceride hydrolysis · lipoproteins · lipase

Enzymatic activity may be assessed accurately and with high sensitivity by measuring changes in surface properties of monomolecular films of appropriate substrates. Changes in surface pressure of triglyceride monolayers resulting from the action of pancreatic lipase have been reported (1–3). Decreases in surface potential and surface radioactivity of phosphatidylcholine monolayers resulted from the action of phospholipase (4–7). In these systems the enzyme catalysis takes place only at the air–water interface with lipid films of defined composition and physical state. The requirements for enzymic reactions involving lipid substrates may be readily determined. The effects of activators and inhibitors on the enzyme, the substrate, and the enzyme–substrate complex may thus be differentiated and properly evaluated.

The initial objective of the series of experiments presented in this report has been to demonstrate that hydrolysis of triglyceride monolayers can be observed with the postheparin lipolytic activity (8). The monolayer techniques appear to be applicable to a study of this complex hydrolytic system and the role of the apoprotein cofactors.

Abbreviations: VLDL, very low density lipoproteins; the apoproteins are identified by the carboxy terminal amino acids (26). ApoLP-Glu, apoLP-Ser-II, and apoLP-Ala refer to the apoproteins from VLDL. ApoLP-Ser-I is the apoprotein from VLDL that is the principal protein constituent of the low density lipoprotein. PHLA, postheparin lipolytic activity.

EXPERIMENTAL PROCEDURES

Materials

Glyceryl tri[1-^{14}C]octanoate, specific activity 8.43 mCi/mmole, and sodium [1-^{14}C]octanoate, specific activity 15.2 mCi/mmole, were purchased from New England Nuclear, Boston, Mass. Glyceryl tri[1-^{14}C]-oleate, specific activity 38 mCi/mmole, and [1-^{14}C]oleic acid, specific activity 57.2 mCi/mmole, were products of Amersham/Searle.

Sodium heparin, 167 units/mg, was obtained from Calbiochem. Triolein, 1,2-diolein, 1,3-diolein, 1-monoolein, and mixed isomers of monoolein were supplied by the Hormel Institute, Austin, Minn. Other chemicals and reagents were purchased from normal commercial sources.

Methods

Postheparin plasma was obtained from normal male subjects, who had fasted overnight, 10 min after intra-

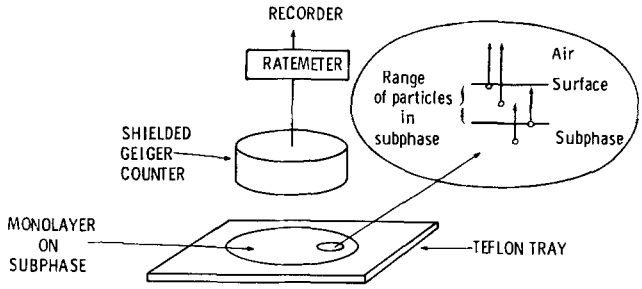

FIG. 1. Measurement of surface radioactivity. Because of the short range of the low energy β particles from ^{14}C, the detector registers only those paritcles originating from the surface monolayer and, to a lesser degree, from the solution just below the film (10). As the radioactive substance is hydrolyzed, water-soluble products leave the surface with a resulting decrease in surface radioactivity.

venous injection of 10 units of heparin/kg of body weight, as described by Fredrickson, Ono, and Davis (9). Heparin was used as the anticoagulant for plasma collection. Occasionally, after 24 hr at 4°C, a precipitate formed which was removed by centrifugation.

The apparatus for determination of surface radioactivity, shown schematically in Fig. 1, consisted of a Nuclear-Chicago model 480 Geiger counter and model 8732 ratemeter with the output recorded continuously on a Barber-Colman PR25 recorder. The sample well, 2.0 × 0.3 cm, 1.0 ml volume, was milled in the center of 0.25-inch Teflon, 3 × 3 inches, fixed to a 0.25-inch aluminum plate by recessed screws at each corner. The surface was sanded with 600-grit carborundum paper to provide a mirror finish. The vessel was placed 1.0–1.5 cm below the Mylar window of the detector. An amount of labeled substrate in hexane was added with a 10-μl Hamilton syringe to the surface of the subphase containing the enzyme, buffer, and other components as desired. A 5-sec time constant was utilized until the signal reached the maximum value, and a 20-sec time constant was used thereafter. Unless otherwise indicated, all assays were performed with the monolayer technique.

The procedure to determine the extent of hydrolysis of emulsions of triolein by postheparin lipolytic activity was a modification of that reported by Huttunen et al. (11). Triolein (8.9 mg) was combined with 5 μCi of glyceryl tri[1-^{14}C]oleate in benzene. The solvent was removed with N_2. Polyvinyl alcohol, 0.5 ml of a 5% (w/v) solution in 1 mM HCl, and 0.49 ml of water were added, and the mixture was subjected to sonic oscillation with the 3/16-inch probe of a Biosonik sonifier for about 1 min, at which time no droplets of triolein were visible and the test tube had warmed to about 40–45°C. Aliquots of freshly prepared emulsions, 0.1 ml, were added to 4 mg of bovine serum albumin, 30 μmoles of Tris–HCl, pH 8.1, and 0.1 ml of normal plasma for a total volume of 0.3 ml. After incubation of this mixture for 30 min at 37°C, aliquots of enzyme and/or water, 0.1 ml, were added for subsequent incubation at 27°C for postheparin lipolytic activity. The samples were processed as described by Huttunen et al. (11), except that Ionac A540 (Matheson Coleman & Bell) was employed as the anion exchanger to retain the labeled fatty acid.

To identify the products of the reaction, the labeled triglyceride was spread on the subphase and enzymic hydrolysis was allowed to proceed to 15–30% of completion, determined by the decrease in surface radioactivity. Enzymatic hydrolysis was terminated by transfer of the total reaction mixture to 2 ml of 2-propanol–heptane–1 N H_2SO_4 40:10:1 (v/v/v) (12). The reaction vessel was washed three times with 1 ml of the solvent mixture. To the combined extracts were added 2 ml of heptane and 3 ml of water. After mixing and centrifugation, 2 ml of the organic phase was removed and dried over anhydrous $MgSO_4$; the solution was then concentrated with N_2 to about 20 μl. The mixture was separated on silica gel G thin-layer plates impregnated with boric acid. Chloroform–acetone 96:4 (v/v) was the solvent (13). The developed plate was divided into 1-cm bands of silica gel which were transferred to vials containing 5 ml of scintillation fluid.

To study the effects of delipidation, aliquots of plasma were frozen in liquid nitrogen and lyophilized. The dried protein was extracted three times with 100 vol of heptane, which was removed by aspiration. Residual solvent was removed with a water pump, followed by evacuation with a mechanical vacuum pump. The samples were reconstituted to the original volume with water. Plasma was extracted with 10% aqueous acetone by the procedure of Fleischer and Fleischer (14) for removal of lipids from bovine heart mitochondria. In one experiment ammonia was included in the extraction solvent to ensure complete removal of acidic phospholipids.

RESULTS

Base-catalyzed hydrolysis of trioctanoin monolayers

The hydrolysis of 400 pmoles of glyceryl tri[1-^{14}C]-octanoate spread as a monomolecular film on the surface of 1 N NaOH is shown in Fig. 2. The reaction displayed first order kinetics under these conditions, in which hydrolysis of the triglyceride and the resulting diglyceride appear to be kinetically equivalent. In separate experiments it was determined by surface pressure measurements that glyceryl monooctanoate does not form a monolayer, i.e., it is soluble in the subphase. A second process was observed when the detector response had decreased about 90%. When 4 nmoles of [1-^{14}C]octanoic acid was placed on the surface of the subphase under identical experimental conditions, a detector response

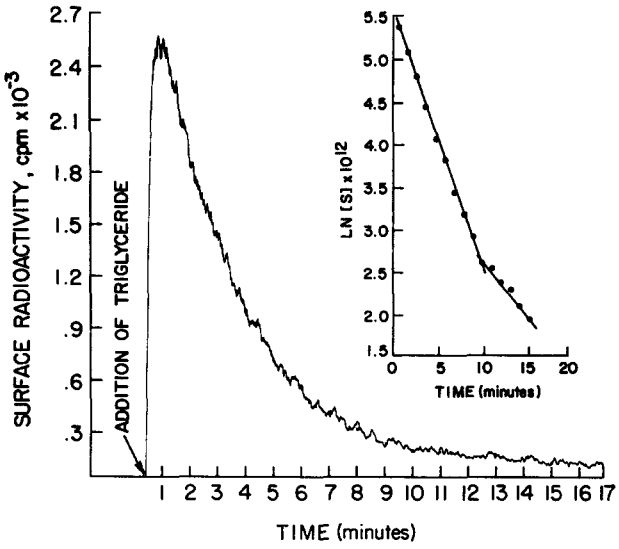

FIG. 2. Hydrolysis of glyceryl tri[1-^{14}C]octanoate monolayers by NaOH. Glyceryl tri[1-^{14}C]octanoate, 400 pmoles in 1 μl of hexane, was applied to the surface, 1.78 cm^2, of 1.0 ml of 1 N NaOH. The break in the first order plot was observed after 90% hydrolysis of the substrate initially on the surface.

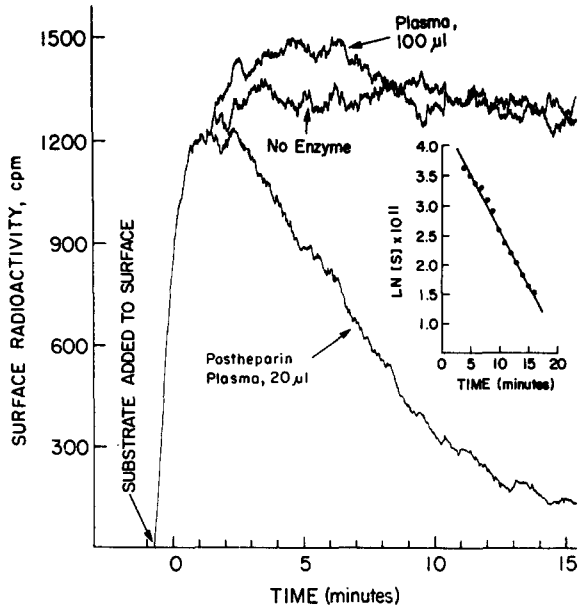

FIG. 3. Hydrolysis of glyceryl tri[1-^{14}C]octanoate monolayers by postheparin lipolytic activity. The reaction mixture contained, in a volume of 1.0 ml, 50 μmoles of Tris-HCl, pH 8.1, 150 μmoles of NaCl, 10 μmoles of $CaCl_2$, and plasma as indicated. After 20–30 min preincubation at room temperature, the mixture was transferred to the reaction vessel, and glyceryl tri[1-^{14}C]octanoate, 400 pmoles, was added to the surface, 1.78 cm^2, in 1.0 μl of hexane. The semilogarithmic plot in the insert depicts the hydrolysis of the monolayer by 20 μl of postheparin plasma.

comparable in magnitude to that observed in Fig. 2 after 10 min was obtained immediately. The rate of decrease of the low levels of radioactivity in both experiments was identical and presumably reflected equilibration of the radioactive material immediately below the surface with the bulk subphase.

Hydrolysis of trioctanoin monolayers by postheparin lipolytic activity

The progress curves which show the effect of normal and postheparin plasma on the disappearance of the glyceryl tri[1-^{14}C]octanoate from the surface in typical experiments are recorded in Fig. 3. When no plasma was present in the subphase, the amount of labeled substrate on the surface was constant for the time period during which the experiments were conducted. Measurement of the surface pressure of trioctanoin at 42.5 pmoles cm^{-2} gave a value of 5.6 dynes cm^{-1}, which indicated that the substrate monolayer was expanded, about 30% of the collapsed film pressure of trioctanoin (2). When plasma taken from subjects before heparin administration was included in the subphase, there was only a slight decrease in surface radioactivity, about 1% of the rate observed when 20 μl of postheparin plasma was included in the subphase. The disappearance of surface radioactivity in the presence of postheparin plasma followed first order kinetics similar to the nonenzymatic hydrolysis by sodium hydroxide. The surface pressure was measured to determine the extent to which endogenous lipids in the plasma contributed to surface pressure. Inclusion of 2, 5, and 10 μl in the subphase gave surface pressure values of 16–18 dynes cm^{-1} on triplicate samples of each. Addition of 400 pmoles of trioctanoin on an area of 1.78 cm^2 increased the surface pressure an additional 5–6 dynes cm^{-1}, a value consistent with an expanded film of endogenous lipids. Larger amounts of plasma produced surface pressure values of 21–23 dynes cm^{-1}. The surface pressure of postheparin plasma was 23 dynes cm^{-1}. At these higher film pressures, addition of the triglyceride substrate caused an increase of only 2–3 dynes cm^{-1}. The surface pressure observed during the time period of the experiments was essentially constant. When the postheparin plasma in the reaction mixture was allowed to remain in the reaction vessel for 30 min before the labeled triglyceride was added to the surface, the rate of disappearance of surface radioactivity was very similar (within 10%) to data obtained when there was no prior incubation. In some experiments, the labeled substrate was added to the surface of the subphase stirred with a magnetic bar, followed by injection of the enzyme into the subphase with a microliter syringe. The time curves for the disappearance of surface radioactivity caused by postheparin plasma under these two conditions were identical.

Hydrolysis of triolein monolayers by postheparin lipolytic activity

Curves essentially identical with those in Fig. 3 were obtained when glyceryl tri[1-^{14}C]oleate was employed

as the substrate in the presence of normal and postheparin plasma. Addition of 100 μg of albumin, 10 μg of apoLP-Ser-II, 10 μg of apoLP-Glu, and 100 μg of apoLP-Ala, alone or in combinations, in the subphase containing 10 μl of postheparin plasma did not cause a significant change in the rate of decrease in surface radioactivity. The decrease in surface radioactivity observed as the result of hydrolysis of a triolein monolayer was anticipated as the consequence of oleic acid binding to the albumin component in plasma. Several experiments were conducted to confirm this supposition. Labeled oleic acid remained at the surface in the absence of plasma. The introduction of 300 μl of normal plasma, 20 μl of postheparin plasma, or 100 μg of fatty acid-poor bovine serum albumin into the stirred subphase (3.0 ml total volume) under an oleic acid monolayer, 42.5 pmoles cm^{-2}, caused an immediate decrease in surface radioactivity. In these experiments, the desorption of oleate from the surface was complete within 30 sec and was not the rate-limiting step.

Identification of reaction products

To establish that hydrolysis of the labeled glyceryl trioleate did, in fact, occur in the presence of postheparin plasma, the products of the reaction were identified by thin-layer chromatography. Three identical reaction mixtures were transferred into 2-propanol–heptane–1 N H_2SO_4 to stop the reaction at 1-min intervals over a 10-min span. The lipids were separated on thin-layer plates of silica gel G containing 2.5% boric acid, using chloroform–acetone 96:4 (v/v). The compounds were visualized with I_2 vapors. The distribution of radioactivity is depicted in Fig. 4. Similar analysis of all lipid samples revealed that with time the proportion of triolein decreased with a concomitant increase in oleic acid, 1,2-diolein, and 2-monoolein. Authentic isomers of the various glycerides were placed on the surfaces of reaction vessels and treated in parallel with the experimental vessels to establish that no isomerization had occurred as the result of experimental procedures. These standard compounds permitted the identification of 1,2-diolein as the primary, if not exclusive, product of triglyceride hydrolysis. As anticipated from the previous studies of lipase specificity, the intermediate 1,2-diglyceride was further degraded to 2-monoolein. An identical experiment with glyceryl tri-[1-^{14}C]octanoate confirmed that the same products were formed from trioctanoin as expected.

Relative rates of hydrolysis of trioctanoin and triolein monolayers

The effect of different amounts of postheparin plasma on triglyceride monolayers is recorded in Figs. 5 and 6. For both trioctanoin and triolein there was considerable deviation from linearity with large amounts of plasma.

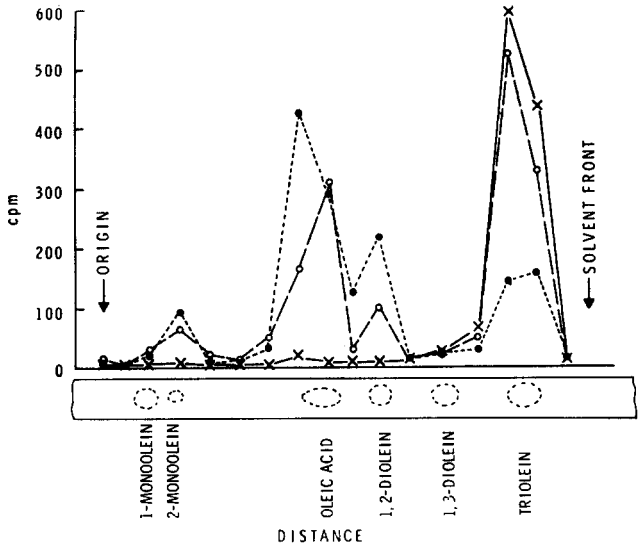

FIG. 4. Identification of reaction products of postheparin lipolytic activity. The hydrolysis of a glyceryl tri[1-^{14}C]oleate monolayer by 20 μl of postheparin plasma was terminated after 20–30% completion by transfer to heptane–2-propanol–1 N H_2SO_4 40:10:1 (v/v/v). The organic phase was dried with Na_2SO_4, reduced in volume, and then applied to silica gel prepared in boric acid. The solvent was chloroform–acetone 96:4 (v/v). The distribution of radioactivity was obtained by incubation with normal plasma (×) and two different postheparin samples (● and ○). A mixture of authentic isomers of the possible reaction products was placed on the surface of a control vessel and processed in parallel to establish that no significant acyl migration had occurred.

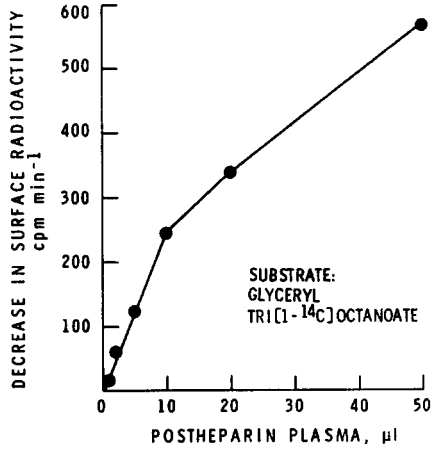

FIG. 5. Effect of postheparin plasma concentration on reaction velocity. Experimental conditions were identical with those described in the legend to Fig. 4. Glyceryl tri[1-^{14}C]octanoate was the substrate.

The kinetic data were obtained from initial rates of decrease in surface radioactivity experiments in which less than 20% of the initial substrate had been hydrolyzed so that the surface concentration of the radioactive substrate was relatively constant. The amount of the intermediate 1,2-diglyceride in the film was determined in the time course experiment described previously and

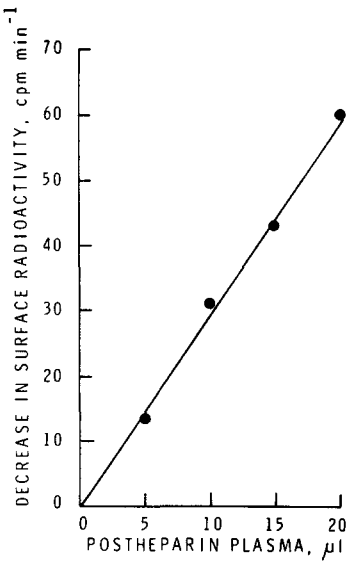

FIG. 6. Effect of postheparin in plasma concentration on reaction velocity. Experimental conditions were identical with those described in the legend to Fig. 4. Glyceryl tri[1-^{14}C]oleate was the substrate.

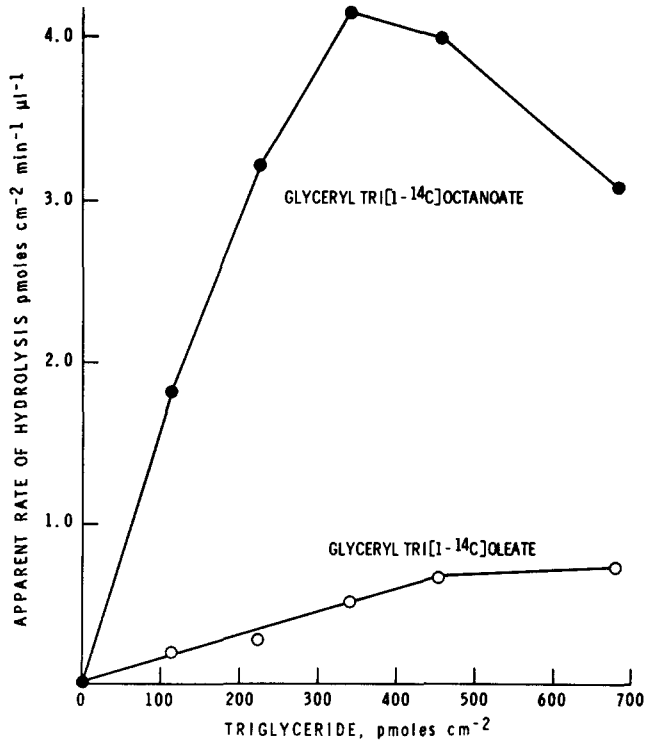

FIG. 7. Relative rates of hydrolysis of trioctanoin and triolein monolayers by postheparin lipolytic activity. The experimental conditions are described in the legend for Fig. 4. The reaction mixture contained 10 μl and 50 μl of postheparin plasma for the hydrolysis of trioctanoin and triolein, respectively. The attenuation of the ratemeter was selected so that the various amounts of radioactive substrate applied to the surface produced an initial recorder response 70–90% of full scale.

did not constitute more than 10% of the film. The contribution arising from the hydrolysis of the diglyceride to the overall rate of decrease in surface radioactivity was not considered significant. Under these conditions it was established by recovery of triolein from reaction products obtained at 1-min intervals that the amount of substrate hydrolyzed was quantitatively related to the decrease in counting rate as a function of time, i.e., the decrease in surface radioactivity and the decrease in the amount of glyceryl tri[1-^{14}C]oleate corresponded and could be accounted for as 1,2-diolein and oleic acid.

Since the hydrolysis of both glyceryl tri[1-^{14}C]octanoate and glyceryl tri[1-^{14}C]oleate was catalyzed by postheparin plasma, it was of interest to compare the relative rates of hydrolysis. As shown in Fig. 7, expanded films of trioctanoin were hydrolyzed about eight times more rapidly than were monomolecular layers of triolein at the same concentration of molecules per unit area. It is assumed that the hydrolysis of both triglycerides is catalyzed by the same lipolytic activity, because previous studies with highly purified lipases (15, 16) have shown that the rate of triglyceride hydrolysis is related to chain length of the acyl moiety and there appears not to be an absolute specificity for any particular triglyceride species. The reason(s) for the decrease in the apparent rate of hydrolysis at the higher concentrations of trioctanoin was not explored, pending purification of the enzyme.

pH dependence of reaction velocity

The profile of the reaction velocity as a function of pH is shown in Fig. 8. The rate of enzymic hydrolysis of trioctanoin was maximal at pH 8.1, with a substantial decrease in rate observed at both lower and higher values.

Hydrolysis of triolein emulsions

Hydrolysis of triglyceride emulsions was determined in initial experiments to establish that the enzymatic activity of interest was present in postheparin plasma and could be analyzed in the sources utilized in this investigation. The release of [1-^{14}C]oleic acid from triolein, as shown in Fig. 9, was linear with increasing amounts of postheparin plasma and was prevented by 2 M NaCl and 10^{-4} M protamine sulfate. The emulsion assay was absolutely dependent on prior equilibration of the labeled triolein with normal plasma before the addition of postheparin plasma. Direct comparison of the monolayer assay with the emulsion assay is somewhat uninformative because of the absence of information about the surface area of the substrate available to the enzyme(s) in the emulsion. The lipolytic activity in 20 μl of postheparin plasma released oleic acid at an initial rate of 150 pmoles/min from a triolein monolayer on a 1.0-ml subphase. In the emulsion reaction mixture of 0.4 ml, 20 μl of postheparin plasma released oleic acid at the rate of 30 pmoles/min under the zero order conditions of the emul-

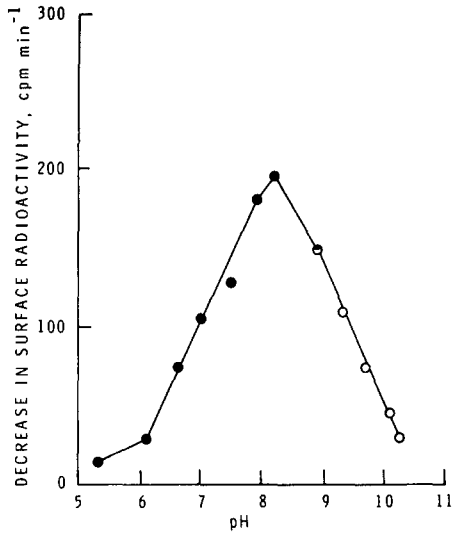

FIG. 8. Reaction velocity as a function of pH. The experimental conditions are described in the legend to Fig. 4. Postheparin plasma (10 μl) was the source of enzyme. Reaction mixtures containing Tris–HCl or acetate buffers are noted as closed circles. Glycine–NaOH buffers were employed at pH values indicated with open circles.

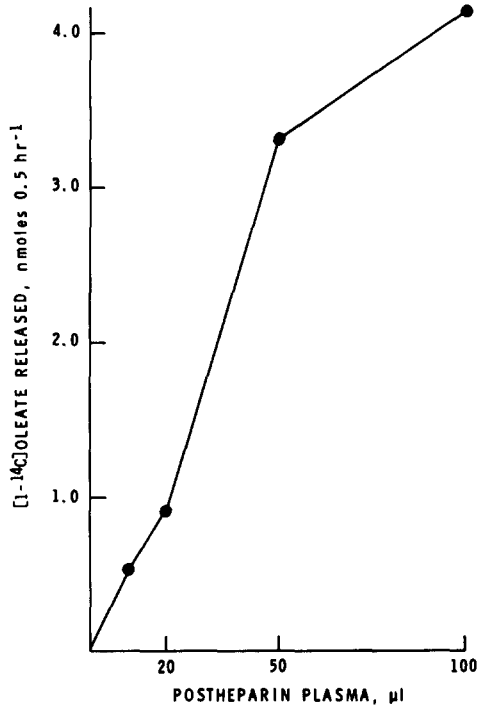

FIG. 9. Hydrolysis of glyceryl tri[1-^{14}C]oleate emulsions. After extraction of the reaction mixture containing the emulsified substrate with heptane–2-propanol–H_2SO_4, the [1-^{14}C]oleic acid in the organic phase was separated from unhydrolyzed triglyceride with an anion exchange resin and counted as described under Methods.

sion. These calculations indicate that the two assay methods are quantitatively comparable. Hydrolysis of L-dioctanoyllecithin by porcine phospholipase A in a monolayer at constant pressure was calculated to be 88 times more rapid than hydrolysis of the same substrate in the micellar state (17).

Inhibitors of postheparin lipolytic activity

The effects of a number of agents that have been described in the literature as inhibitors of lipoprotein lipase are tabulated in Table 1. The hydrolysis of monomolecular layers of both trioctanoin and triolein by postheparin lipolytic activity shows the characteristic inhibition pattern (8) described for the action of this enzymic activity on triglyceride emulsions or chylomicrons. When the reaction mixtures containing the various inhibitors, except 2 M NaCl and 0.1 M NaF, were assayed immediately after preparation, there was only slight and variable inhibition of the hydrolysis. The variability in the degree of inhibition has been observed with emulsified substrates also (18, 19). The addition of sodium heparin (0.1, 1.0, and 10 units) to the reaction mixture with 10 μl of postheparin plasma produced no greater than 50% increase in the rate of hydrolysis of either substrate. The response to Ca^{2+} was variable, producing in some experiments up to a 30% stimulation of the rate of hydrolysis. To eliminate this variability, 10 mM $CaCl_2$ was routinely included in the reaction mixture.

Effect of endogenous triglycerides

The effect of endogenous triglycerides on the reaction rate was investigated in two kinds of experiments. The addition of equal volumes of normal plasma and postheparin plasma did not reduce the rate of hydrolysis. Removal of neutral lipids from plasma under anhydrous conditions was accomplished by heptane extraction after the samples had been frozen in liquid nitrogen and lyophilized. This procedure removed the bulk of the cholesterol, cholesteryl esters, and triglycerides as shown by thin-layer chromatography. Full activity (> 90%) toward both trioctanoin and triolein was retained. It was concluded that the hydrolysis of the film was relatively insensitive to normal levels of endogenous lipids.

TABLE 1. Inhibition of postheparin lipolytic activity

Inhibitor	% of Control
None	(100)
NaCl, 2 M	45
Protamine sulfate, 10^{-4} M	30
$Na_4P_2O_7$, 10 mM	14
NaF, 0.1 M	11

The reaction mixture contained 10 μl of postheparin plasma, 50 μmoles of Tris–HCl, pH 8.1, 150 μmoles of NaCl, 10 μmoles of $CaCl_2$, and the indicated inhibitor in a total volume of 1.0 ml. After 1 hr at room temperature, the reaction mixture was transferred to the Teflon tray, and the substrate, 400 pmoles of glyceryl tri[1-^{14}C]octanoate, was added to the surface. The average rate of hydrolysis for the controls was 2.7 pmoles cm^{-2} μl^{-1} min^{-1}. The values are averages of three experiments.

Efforts to retain hydrolytic activity after removal of lipids from lyophilized postheparin plasma by extraction with anhydrous chloroform–methanol 2:1 (v/v) or with 90% acetone were unsuccessful. No activity was regained after incubation of any of the delipidated samples with albumin–dioleoyl phosphatidylcholine emulsions or with normal plasma. Experiments to provide information about the possible phospholipid requirement require purified enzyme preparations.

DISCUSSION

The conversion of triglycerides in mammalian plasma to glycerol and fatty acids is greatly enhanced after the administration of heparin (8). A multiplicity of hydrolytic activities have been identified in postheparin plasma (20–25). Emulsions of triglycerides have been employed extensively in these studies. Determination of these enzyme activities depends to a variable degree on subsequent hydrolysis of the initial reaction products. The apparent rates of hydrolysis and substrate specificities observed may be more directly related to the stability of the emulsions and/or to the effects of the emulsifying agents on various components of the system than to actual lipid–protein interaction and subsequent catalysis by the enzyme. These ambiguities introduce uncertainty in the assessment of enzyme specificity, kinetic behavior, and cofactor requirements. Some of these problems, principally those concerned with the concentration and physical state of the substrate available to the enzyme, can be avoided with monolayer systems.

The measurement of enzymic activity by changes in surface radioactivity of triglyceride films can produce useful information. With due care, initial reaction rates can be determined in short time intervals in which no more than 20% of the triglyceride substrate has been hydrolyzed. With this limitation, effects of changes in the concentration, composition, and surface pressure of the film can be minimized. A number of considerations regarding the hydrolysis of triglyceride monolayers by postheparin lipolytic activity require clarification. These include the dependence of the reaction velocity on the surface pressure of the film, the effect on triglyceride hydrolysis of constant surface pressure maintained by endogenous lipids and/or lipoproteins, and the relative rates of hydrolysis of triglyceride and 1,2-diglyceride by the triglyceride lipase in order to understand the observed first order kinetics. In samples from which the neutral lipids had been removed, the absence of hydrolytic activity might result from inhibition by partially delipidated low density lipoprotein (22).

The results described in this paper illustrate the applicability of monolayer techniques to the postheparin lipolytic system. Comparison of results obtained with both monolayer and emulsion assay procedures reveals the absence of this hydrolytic activity in normal human plasma, its appearance after heparin administration, the linearity of response to different amounts of plasma, the relative insensitivity of the monolayer assay technique to interference by normal concentrations of endogenous lipids in the subphase, and characteristic inhibition of the postheparin lipolytic activity. The monolayer technique also provides a continuous progress curve of the reaction from which kinetic information is easily obtained, in contrast to the laborious, time-consuming, emulsion assays. The monolayer assay technique provides information that is complementary to that obtained by methods which employ emulsified substrates.

Substrates with specifically labeled acyl moieties in conjunction with the monolayer techniques should permit the unequivocal identification and differentiation of the various hydrolytic activities reported to be released by heparin administration. The kinetic parameters now accessible should allow the relative contributions of the various enzymic species to the metabolism of plasma lipoproteins to be assessed. Experiments to achieve these objectives are in progress.

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