Acylation of *sn*-glycerol 3-phosphate by cell fractions of rat liver

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ABSTRACT The esterification of *sn*-glycerol 3-(dihydrogen phosphate) with long-chain fatty acids by rat liver microsomal preparations has been studied. A newly modified spectrophotometric assay for glycerolphosphate acyltransferase (GP-acyltransferase) compared favorably with other assay methods, including measurement of the incorporation of *sn*-glycerol-^{14}C 3-(dihydrogen phosphate) into glycerolipids. Cofactor requirements, preliminary kinetic constants, and optimum pH were determined. The product of the reaction was identified as monoacylglycerophosphate by thin-layer chromatography.

Albumin activated GP-acyltransferase at low concentrations of acyl CoA but was inhibitory at higher concentrations. Serum β-lipoprotein also caused activation of GP-acyltransferase. The effect of albumin could not be attributed to binding of substrate or fatty acids or the provision of metal ions.

Diacylglycerophosphate, cytidine triphosphate, sulfhydryl binding agents, and sodium palmitate were identified as inhibitors of microsomal GP-acyltransferase. The physiological significance of these inhibitors remains to be established.

KEY WORDS *sn*-glycerol 3-phosphate · acyltransferase · modified spectrophotometric assay · activation · inhibition · albumin · serum proteins · metal ions

THE ESTERIFICATION of *sn*-glycerol 3-(dihydrogen phosphate) by long-chain fatty acids has been proposed as a possible regulatory step in hepatic lipogenesis (1–4). This reaction is mediated by the microsomal enzyme glycerolphosphate acyltransferase (EC 2.3.1.15) (GP-acyltransferase) (5). Recent studies have shown that diets of high carbohydrate content may increase the rate of esterification by rat liver homogenates (1). An increase in hepatic GP-acyltransferase induced by the high carbohydrate diets may participate in this effect (1).

Previous studies of this enzyme in rat liver (6–8) have not established the product of the reaction nor the reliability of the assay method. Furthermore, there is little information on the effects of cofactors, substrates, and possible activators or inhibitors on reaction rate.

The measurement of GP-acyltransferase by a simple spectrophotometric assay (6) has therefore been modified to provide a reliable and rapid measure of this activity in rat liver microsomes. The product of the reaction has been identified, chromatographically, as monoacylglycerophosphate. The effects of several potentially important activators and inhibitors on reaction rates were measured.

METHODS AND MATERIALS

All lipid standards were obtained from The Hormel Institute, Austin, Minn., or Applied Science Laboratories Inc., State College, Pa. 1,2-diacyl-*sn*-glycerol 3-phosphate was a gift of W. E. M. Lands, and 1-acyl-*sn*-glycerol-^{14}C 3-phosphate a gift of J. M. Johnston. Silica Gel G and H were products of Brinkmann Instruments, Inc. Palmitoyl CoA, oleoyl CoA, and linoleoyl CoA were prepared by the method of Seubert (9) and identified by ultraviolet absorption spectrum (10) and hydroxamate formation (1, 11). Coenzyme A was obtained from Sigma Chemical Company and Boehringer Mannheim, Inc. Fatty acid-poor albumin was obtained from Pentex, Inc., Kankakee, Ill., and other human serum protein fractions from Mann Research Lab. Inc, New York. *sn*-Glycerol-^{14}C 3-phosphate was obtained from Nuclear Research Chemicals Inc., Orlando, Fla., and its purity determined by paper chromatography

Abbreviations: GP-acyltransferase, glycerophosphate acyltransferase; DTN, 5,5′-dithio-bis-(2-nitrobenzoic acid); ACP, acyl carrier protein; TLC, thin-layer chromatography.

(12). All other materials were obtained from well-known commercial sources.

Preparation of Microsomes

Microsomes were prepared from the liver of adult male rats (150–250 g) that had been fed laboratory chow. The liver was homogenized briefly in 4 volumes of iced 0.25 M sucrose containing 0.01 M Tris–HCl buffer pH 6.5 and 1 mg/liter of D,L-α-tocopherol, hereafter called sucrose–Tris buffer. Cell debris was removed by centrifugation at 400 *g* for 10 min. Mitochondria were removed by sedimentation twice at 16,000 *g* for 15 min. The microsomal fraction was prepared by centrifugation at 105,000 *g* for 30 min in a Spinco model L ultracentrifuge. The microsomes were washed and resuspended in the same buffer. This preparation was homogenized and resedimented at 105,000 *g* for 30 min. Microsomes were resuspended in $^1/_3$ the original volume of sucrose–Tris buffer and cautiously homogenized by hand. The identity of the microsomal preparation was verified by electron microscopy. Storage at 4°C for 3–4 days resulted in a gradual decrease in GP-acyltransferase activity.

Lipid Determinations

The purity of all lipid standards and reagents was determined by thin-layer chromatography (TLC) in several systems. Glycerides were separated on Silica Gel G plates in *n*-hexane–ether–glacial acetic acid 73:25:2 or by the system of Pieringer and Kunnes (13). Phospholipids were separated and identified on Silica Gel H plates by the methods of Artom (14), Skipski, Peterson, and Barclay (15), and Johnston, Rao, Lowe, and Schwartz (16). Diacylglycerophosphate and monoacylglycerophosphate were separated with each of these solvent systems and the system of Lands and Hart (17). Samples and standards were identified by exposure to iodine vapor. Radioactive samples were identified by comparison with known standards and then scraped from the thin-layer plates directly into liquid scintillation counting bottles. Elution of the lipids from the silica gel prior to radioactivity determination or addition of a thixotropic gel to the counting mixture containing silica gel did not alter the radioactivity determinations.

The radioactive lipid product was hydrolyzed with 0.2 M KOH (18) and the water-soluble derivatives were separated by paper chromatography (19). The product was also exposed to hydrolysis with phospholipase C (phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3) (20) and phospholipase D (phosphatidylcholine phosphatidehydrolase, EC 3.1.4.4) (21).

Assay Procedures

GP-acyltransferase was measured by a modification of previous methods (6, 8). The glycerophosphate-dependent release of free CoA from palmitoyl CoA was a measure of enzyme activity. The standard assay was performed in two 10 × 70 mm test tubes which both contained 0.08 M Tris-HCl pH 6.5, 0.015 M dithiothreitol, 2.5 mg of fatty acid-poor albumin, and 150 mμmoles of palmitoyl CoA in a volume of 0.22 ml. 0.02 ml of 1 M *rac*-glycerol 3-phosphate in 0.5 M Tris-HCl pH 6.5 was added to the control. The mixture was preincubated at 35°C for 5 min, and the reaction was started by adding 0.1 ml of microsomal preparation (0.2–1.0 mg of protein per ml) to both assays. The reaction was stopped at 15 min by the addition of 1 ml of 3.5% (w/v) perchloric acid. The mixture was allowed to stand in ice for 30 min, and then centrifuged at 400 *g* for 10 min. Palmitoyl CoA was precipitated by this procedure, but free CoA remained in solution (6). The ultraviolet spectrum of the perchlorate supernate was identical to that of authentic CoA in 3.5% perchlorate. The difference in optical density at 260 mμ between the assays performed in the presence and absence of glycerophosphate was a measure of GP-acyltransferase activity. A molar extinction coefficient of 16,000 at 260 mμ was used for CoA in 3.5% perchloric acid (22). Units are defined as μmoles × 10^{-2} of CoA released per 15 min. All determinations were performed in duplicate.

The release of free CoA in this reaction was also determined by reaction of the product with 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTN) as described by Ellman (23). 1 ml of water was added to the reaction mixture after incubation for 15 min at 37°C and the reaction was immediately stopped by boiling for 5 min. The precipitate was removed by centrifugation, and an aliquot of the supernatant fraction was added to 2.5 × 10^{-3} M DTN. The optical density at 412 mμ was recorded. A molar extinction coefficient of 13,600 at 412 mμ was used for the DTN derivative (7).

GP-acyltransferase activity was also measured by the rate of incorporation of *sn*-glycerol-^{14}C 3-phosphate into lipids. The assay conditions were identical with those of the standard assay except that 0.4 μc of *sn*-glycerol-^{14}C 3-phosphate (10 mc/mmole) and 0.02 ml of 1 M *rac*-glycerol 3-phosphate were added to the reaction mixture. The reaction was started by the addition of microsomes and stopped with 20 volumes of chloroform–methanol 2:1. Lipids were extracted and washed by the method of Folch, Lees, and Sloane Stanley (24). Radioactivity of the total extract and of particular glycerolipids was determined by liquid scintillation spectrometry. The glycerolipids were separated and identified by the TLC techniques described above.

The protein concentration of cell fractions was determined by the method of Lowry, Rosebrough, Farr, and Randall (25). Microsomal preparations were hydrolyzed with 1 N NaOH prior to protein determination.

RESULTS

Measurement of GP-acyltransferase Activity and Identification of Product

The rate of GP-acyltransferase as measured by the standard assay was shown to be linear for at least 15 min (Fig. 1). Acyl-CoA hydrolase activity can be determined simultaneously by measurement of the release of CoA from palmitoyl CoA in the absence of *sn*-glycerol 3-phosphate. Hydrolase activity was approximately 50% of the corresponding GP-acyltransferase activity.

The reaction rate was directly proportional to microsomal concentration over a limited range (Fig. 2). The apparent inhibition of GP-acyltransferase activity at high microsomal concentration was observed in all preparations, regardless of rat diet or method of assay. Microsomal concentration was chosen in all subsequent studies so as to lie within the linear range.

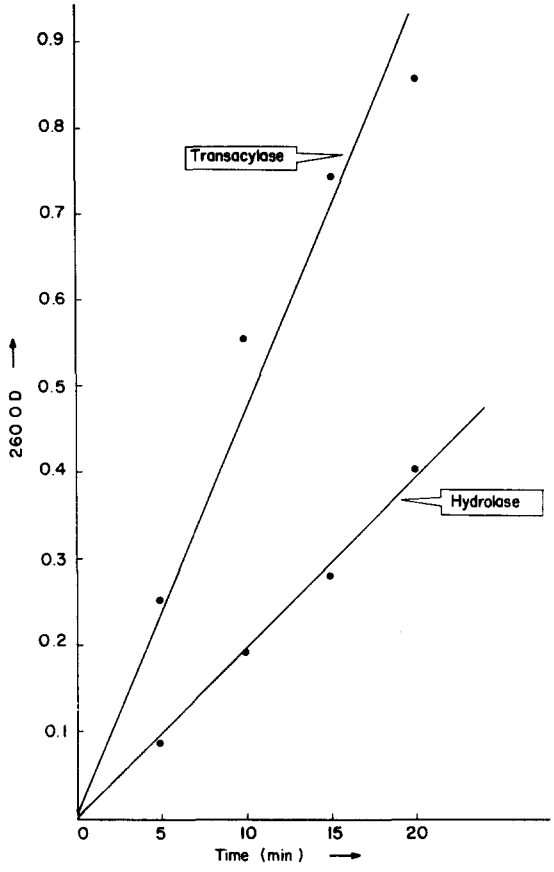

FIG. 1. GP-acyltransferase activity (transacylase) in rat liver microsomes as measured by the standard assay. The activity was linear for 20 min. The palmitoyl-CoA deacylase activity (hydrolase) was measured simultaneously as the release of CoA in the absence of *sn*-glycerol 3-phosphate. Reaction rate is recorded as the glycerophosphate-produced optical density (OD) at 260 mμ for the GP-acyltransferase and as the total OD at 260 mμ in the absence of glycerophosphate for the palmitoyl CoA deacylase. Values are means of duplicate determinations at each time period.

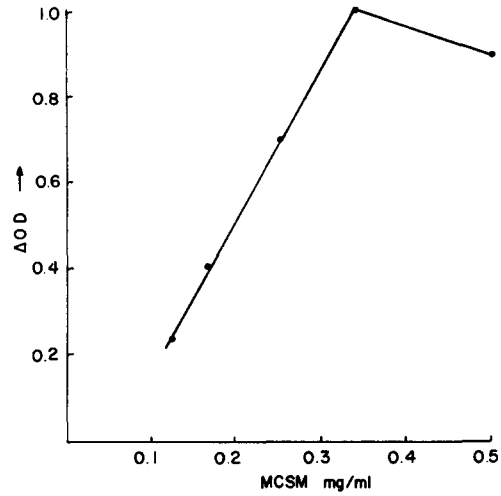

FIG. 2. GP-acyltransferase activity as measured by the standard assay. The microsomal (MCSM) concentrations refer to the final concentration of protein in the standard assay volume of 0.34 ml. Reaction rate is recorded as optical density (OD) at 260 mμ.

The optimum pH for GP-acyltransferase activity in the standard assay was 6.5 (Fig. 3). A similar pH optimum of 6.5 was noted when oleoyl or linoleoyl CoA replaced palmitoyl CoA as the substrate.

The effect of omitting various components of the standard assay is shown in Table 1. Dithiothreitol was not required for the reaction, but resulted in an increased reaction rate.

Oleoyl CoA and linoleoyl CoA were active substrates; thus the reaction is not specific for palmitoyl CoA. Maximum activity in the standard assay was obtained with a concentration of 0.45 mM for palmitoyl CoA and 0.68 mM for oleoyl or linoleoyl GoA. The acyl CoA derivatives were inhibitory at higher concentrations.

The release of CoA was measured in the standard assay by the increase in optical density at 260 mμ. This method was compared with measurement of the CoA released by reaction with DTN at completion of the assay. Both methods gave directly proportional results when the concentration of microsomes was varied over the optimal

TABLE 1 EFFECT OF OMITTING REAGENTS FROM THE STANDARD ASSAY ON REACTION RATE

Omissions	Relative Activity
None	1.00
Palmitoyl CoA	0.00
Palmitoyl CoA (hydrolysis)	0.01
Microsomes	0.03
Dithiothreitol	0.80
rac-Glycerol 3-phosphate	0.00

Palmitoyl CoA was omitted or hydrolyzed with 1 N NaOH (hydrolysis) prior to incubation. The final volume of all assays was the same.

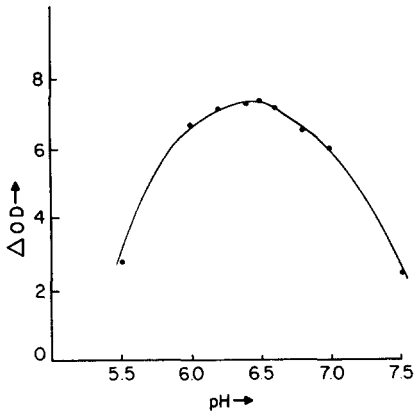

FIG. 3. Plot of reaction velocity against pH for GP-acyltransferase measured by the standard assay.

range. The release of CoA during the assay could be measured by continuously recording optical density at 412 mμ in the presence of 10^{-4} M DTN. Unfortunately, this was an inadequate routine assay because of the inhibitory effect of DTN and the reactivity of dithiothreitol with DTN.

CoA was incubated with microsomes in the absence of palmitoyl CoA under the usual assay conditions. The reaction mixture contained 2.5 μmoles of CoA in the presence or absence of 6.0×10^{-5} M sodium palmitate. No decrease in optical density at 260 mμ or in material reacting with DTN was observed over the 20 min time period.

The assay of GP-acyltransferase by measurement of CoA release was correlated with the rate of glycerophosphate-^{14}C incorporation into glycerolipids. This assay was performed in the same way as the standard assay except that the reaction contained *sn*-glycerol-^{14}C 3-phosphate and was stopped by the addition of chloroform–methanol. Lipids were extracted and separated by TLC. Incorporation of isotope into total lipids was linear with respect to time and microsomal concentration. The latter relationship is shown in Fig. 4. No incorporation occurred in the absence of palmitoyl CoA.

A comparison of GP-acyltransferase activity measured by CoA release and by isotope incorporation into glycerolipids is shown for one experiment in Table 2. The mean ratio of μmoles of CoA released to μmoles of *sn*-glycerol-^{14}C 3-phosphate incorporated into lipids in several experiments was 0.98 ± 0.05. The molar ratio of almost unity suggested that monoacylglycerophosphate was the probable product of the reaction measured.

The product was identified by TLC of the radioactive glycerolipids at the end of the reaction. Four different solvent systems for identification of phospholipids were used (14–17) with diacylglycerophosphate-^{14}C and monoacylglycerophosphate-^{14}C as standards. The major radioactive product was chromatographically identical with monoacylglycerophosphate, as shown in Fig. 5. Results with the two solvent systems that allow the widest separation of monoacylglycerophosphate and diacylglycerophosphate are shown. No significant radio-

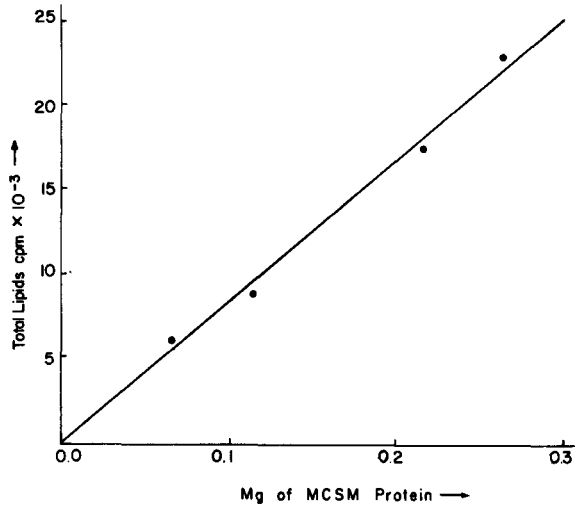

FIG. 4. Incorporation of *sn*-glycerol-^{14}C 3-phosphate into lipids under the conditions of the standard assay. The concentration of microsomes (MCSM) is recorded in mg of protein per assay.

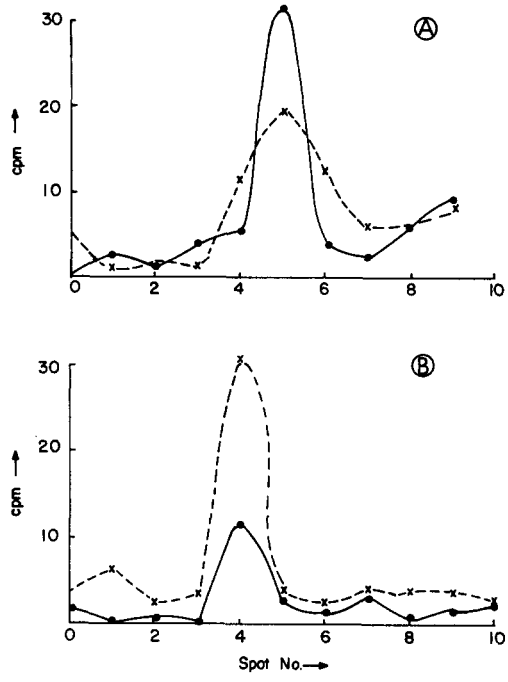

FIG. 5. The radioactive spots when *sn*-glycerol-^{14}C 3-phosphate was used as a substrate and the reaction product chromatographed on Silica Gel H. The solvent systems in A (17) and B (15) were found to give the clearest separation of monoacyl- and diacylglycerophosphate. The radioactive monoacylglycerophosphate standard is indicated by the dashed line, and the radioactive reaction product by the solid line. Spot number *0* is the origin, and *10* is the solvent front. Three times as much radioactive product was chromatographed on the A solvent plate. Diacyl glycerophosphate was identified in spot *9* in both solvents.

TABLE 2 RELEASE OF FREE CoA MEASURED BY THE STANDARD ASSAY COMPARED TO THE INCORPORATION OF *sn*-GLYCEROL-^{14}C 3-PHOSPHATE (L-α-GP-^{14}C) INTO TOTAL LIPIDS

Microsome Protein	L-α-GP-^{14}C to Lipids	CoA Released	Ratio CoA/L-α-GP
mg/ml	*μmoles/10 min*		
0.09	0.0152	0.0126	0.829
0.12	0.0198	0.0215	1.085
0.18	0.0210	0.0222	1.054
0.30	0.0422	0.0398	0.944
0.45	0.0522	0.0510	0.978

The concentration of *sn*-glycerol 3-phosphate (L-α-GP-^{14}C) was 0.03 M in each assay. CoA was measured spectrophotometrically with a molar extinction coefficient, ϵ_{260}, of 16,000. Microsomal concentration in the first column refers to the final protein concentration in the standard assay volume of 0.34 ml.

TABLE 3 EFFECT OF ADDED PROTEIN ON REACTION RATE OF GP-ACYLTRANSFERASE

Protein Addition	Protein Concentration	Relative Activity
None	0	1.0
Albumin	7.3	5.1
Albumin (dialyzed vs. EDTA)	7.3	5.0
β-Lipoprotein	14.6	3.6
γ-Globulin	7.3	3.6
β-Globulin	7.3	2.3
Fibrinogen	3.7	0.7

Proteins were added at a concentration which gave maximal stimulation. The protein concentration is recorded in mg of protein per ml of the final reaction mixture. In one assay, albumin was dialyzed against 100 volumes of 10^{-2} M EDTA for 18 hr with two changes of buffer and then redialyzed against 0.375 M Tris–HCl buffer, pH 6.5.

activity could be detected in diacylglycerophosphate or in other glycerolipid fractions.

The radioactive product was hydrolyzed with 0.2 M KOH as described by Maruo and Benson (18). Both the total lipid extract and the radioactive phospholipid scraped from thin-layer chromatograms were hydrolyzed. All of the radioactivity in the water-soluble hydrolysate was identified as *sn*-glycerol 3-phosphate by paper chromatography (19). Incubation of the lipid extracts containing the radioactive product with phospholipase D or phospholipase C did not change the mobility of the product in the several TLC solvents. These results are compatible with the proposed identification of the product as monoacylglycerophosphate.

The effects of changes in substrate concentration on reaction rate are shown in Fig. 6. Tentative Michaelis constants calculated from these data are $K_m = 6.7 \times 10^{-3}$ M for *sn*-glycerol 3-phosphate and $K_m = 1.1 \times 10^{-4}$ M for palmitoyl CoA. When oleoyl CoA was substituted for palmitoyl CoA, a K_m of 2.6×10^{-4} M was obtained. The substrate concentrations which gave half maximum rates are approximately equal to the reported physiological concentrations of *sn*-glycerol 3-phosphate and of long-chain acyl CoA derivatives in liver (5, 26, 27).

The comparative specific activity of GP-acyltransferase in mitochondria, microsomes, and particle-free supernate was measured by the spectrophotometric assay. The highest specific activity was noted in the microsomal fraction (13.6 units/mg of protein). Mitochondria (4.6 units/mg of protein) and the supernatant fraction (5.8 units/mg of protein) were nearly equal in specific activity.

Effects of Activators and Inhibitors

The effect of albumin on GP-acyltransferase activity is shown in Fig. 7. Maximum activation was achieved at albumin concentrations of 7.3 mg/ml; higher levels were inhibitory. A similar activating effect of albumin was obtained when *sn*-glycerol-^{14}C 3-phosphate was used to measure the reaction. The activation by albumin was not changed by dialysis of the albumin for 18 hr against 100 volumes of 0.1 M Na_2 EDTA, as shown in Table 3. Removal of trace amounts of fatty acids from albumin by charcoal did not alter the activating effect.

The effect of increasing palmitoyl CoA concentration on reaction rate is also shown in Fig. 7. The concentration of palmitoyl CoA that gave maximum GP-acyltransferase activity and the concentration causing inhibition of activity were both reduced by albumin.

The effects of various other serum proteins on GP-acyltransferase activity are shown in Table 3. Serum β-lipoprotein, β-globulin, and γ-globulin increased activity, but fibrinogen was inhibitory.

Binding of palmitoyl CoA by these various proteins was evaluated by incubation of the protein with palmitoyl CoA in the absence of microsomes. Protein was added in the concentration that gave maximum GP-acyltransferase stimulation. Protein was precipitated by boiling and the ultraviolet absorption spectrum of the supernate was determined. Appropriate controls containing protein but no palmitoyl CoA were studied simultaneously. The optical densities at 260 mμ of these control samples were subtracted from the values obtained in the presence of palmitoyl CoA. Palmitoyl CoA was removed from micellar solution by precipitation of all proteins except fibrinogen. There was no apparent quantitative association between an activating effect of serum proteins and their ability to bind the substrate, palmitoyl CoA.

The addition of inactivated microsomes at concentrations used in the standard assay removed approximately half of the palmitoyl CoA from the dilute solution.

The effect of soluble cellular proteins on GP-acyltransferase activity was compared with the activating effect of albumin. A marked inhibition of activity was

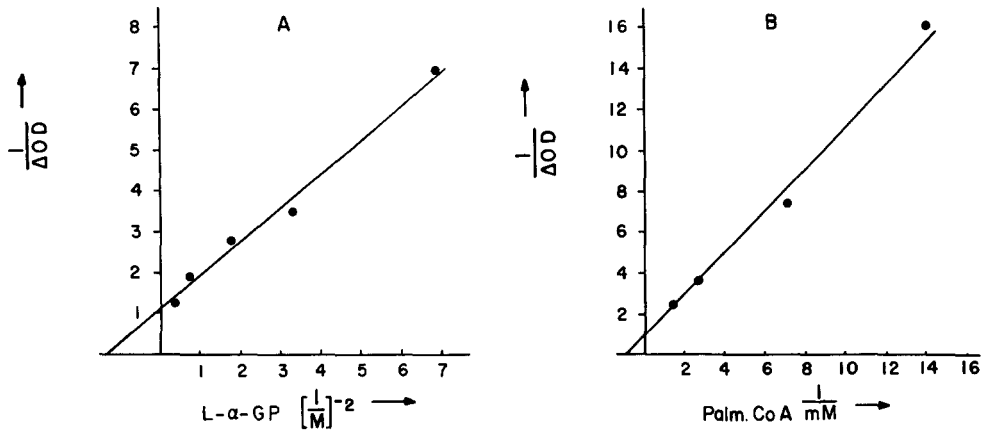

FIG. 6. Double reciprocal plots of reaction velocity against substrate concentration. *sn*-Glycerol 3-phosphate (L-α-GP) was the substrate varied in A, and palmitoyl CoA (Palm. CoA) in B. Assays were performed under optimum conditions except for the appropriate substrate.

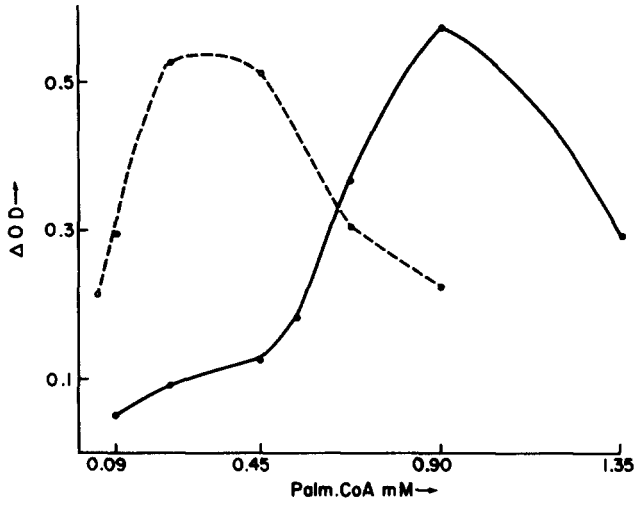

FIG. 7. Effect of albumin on reaction velocity at various concentrations of palmitoyl CoA (Palm. CoA). The standard assay was used. Both the concentration of palmitoyl CoA causing maximum GP-acyltransferase activity and the concentration causing inhibition of activity were reduced in the presence of albumin. Broken line, with albumin; solid line, no albumin.

noted when the particle-free supernate replaced albumin in the standard assay (Table 4). However, a 20% increase in acyl hydrolase activity occurred in the presence of the supernatant fraction. Several protein fractions were separated from the particle-free supernate by ammonium sulfate precipitation. All were inhibitory to GP-acyltransferase. The soluble protein remaining after 85% saturation with ammonium sulfate was least inhibitory to GP-acyltransferase but caused the greatest increase in acyl hydrolase activity.

The effects of the addition of metal ions on GP-acyltransferase were studied. As shown in Table 5, $MgCl_2$ and $MnCl_2$ inhibited activity. Although EDTA caused some inhibition of activity, extensive dialysis of microsomes or albumin against buffers containing EDTA did not alter activity in the standard assay. Thus, a requirement for metal ion addition has not been established.

The effects of various potential inhibitors of GP-acyltransferase were evaluated. Table 6 shows the effect of several lipid additions on reaction rate. The addition of diacylglycerophosphate and sodium palmitate resulted in a significant inhibition of acyltransferase activity. The concentrations of lipids other than palmitate must be considered approximate because of their low solubility in the medium. Homogeneous dispersion was achieved by the method described in Table 6.

Cytidine triphosphate, 10^{-3} M, caused 50% inhibition of GP-acyltransferase when measured by the standard assay or by *sn*-glycerol-^{14}C 3-phosphate incorporation into lipid. Adenosine triphosphate had no effect on GP-acyltransferase activity by either assay.

Two sulfhydryl inhibiting agents reduced GP-acyltransferase activity in the presence of dithiothreitol

TABLE 4 COMPARISON OF SOLUBLE FRACTIONS IN THE ACTIVATION OF GP-ACYLTRANSFERASE

Protein Addition	GP-Acyltransferase
	units
None	6.15
Cell supernate (dialyzed)	0.64
Ammonium sulfate (0–65)	1.81
Ammonium sulfate (65–85)	2.81
Ammonium sulfate (>85)	4.60

The microsomes were identical in all assays. The 105,000 *g* supernatant fraction was dialyzed for 18 hr against 100 volumes of 0.1 M Tris–HCl, pH 6.5. Ammonium sulfate was added to this fraction and the material precipitating between 0 and 65% saturation (0–65) and between 65 and 85% saturation (65–85) was dissolved in 0.1 M Tris–HCl, pH 6.5. These fractions and the 85% saturation supernate (>85) were dialyzed against 100 volumes of the same buffer for 18 hr. The volume was adjusted to give a final protein concentration equal to that of albumin in the standard assay.

TABLE 5 EFFECT OF METAL IONS ON GP-ACYLTRANSFERASE ACTIVITY

Addition	Relative Activity
None	1.0
$MgCl_2$ (1.2×10^{-2} M)	0.6
$MgCl_2$ (2.4×10^{-2} M)	0.4
$MgCl_2$ (3.6×10^{-2} M)	0.3
$MgCl_2$ (4.8×10^{-2} M)	0.2
$MgCl_2$ (6.0×10^{-2} M)	0.1
KCl (1.2×10^{-2} M)	1.0
$CaCl_2$ (1.2×10^{-2} M)	1.0
$MnCl_2$ (1.2×10^{-2} M)	0.8
EDTA (1.2×10^{-2} M)	0.8

The standard assay was conducted in the presence of albumin; various amounts of $MgCl_2$, $CaCl_2$, $MnCl_2$, KCl, and EDTA were added (final concentration shown).

TABLE 6 EFFECT OF VARIOUS LIPIDS

Additions	Molarity	Relative Activity
None		1.0
Lysolecithin	10^{-3}	1.0
Lecithin	5.5×10^{-4}	0.9
Diacylglycerophosphate	6.2×10^{-4}	0.6
Dipalmitin	8.8×10^{-5}	0.9
Tripalmitin	6.2×10^{-5}	0.9
Sodium palmitate	1.5×10^{-4}	0.7
Sodium palmitate	4.0×10^{-4}	0.4

The lipids were added to reaction tubes in benzene, the solvent was evaporated under N_2, and the reaction mixture was added. Tubes were shaken until a homogeneous dispersion of lipid material was obtained. Mixtures containing sodium palmitate were clear.

TABLE 7 EFFECT OF SULFHYDRYL INHIBITING AGENTS

Additions	Relative Activity
None	1.0
Iodoacetamide	1.0
N-Ethylmaleimide	0.5
Dithionitrobenzene	0.6

The inhibitors were added to give a final concentration of 2 μmoles/ml in the standard assay.

(Table 7). These agents did not inhibit palmitoyl-CoA hydrolase activity at the concentrations studied. The omission of dithiothreitol from the standard assay mixture resulted in a 20% decrease in GP-acyltransferase reaction rate.

DISCUSSION

The acylation of *sn*-glycerol 3-phosphate is the first specific reaction in hepatic glycerolipid synthesis. Changes in the rate of this reaction may participate in the regulation of fatty acid synthesis by removing inhibitory long-chain acyl CoA derivatives (3, 4, 28). Recent studies have suggested that alterations in diet may result in changes in the activity of this reaction in rat liver (2). Therefore, present evidence suggests a possible role for this reaction in the regulation of hepatic fatty acid and glycerolipid synthesis under various physiological conditions.

Initial studies of GP-acyltransferase activity by previous methods (6, 8) gave inconsistent results. Various changes, including elimination of the phosphate buffer system, careful adjustment of pH, preincubation of palmitoyl CoA and albumin in the presence of *sn*-glycerol 3-phosphate, and the use of microsomes to start the reaction improve the reliability of the assay. These and other changes resulted in an apparently reproducible and specific spectrophotometric assay.

Determination of CoA release by measurement of optical density at 260 mμ was directly proportional to the simultaneous measurement of CoA with DTN. Thus, changes in optical density at 260 mμ accurately reflect the rate at which CoA or some direct derivative is released from palmitoyl CoA. CoA was not reutilized or bound to protein under the conditions of the standard assay.

The reliability of the assay as a measure of *sn*-glycerol 3-phosphate esterification was verified further by measurement of *sn*-glycerol-^{14}C 3-phosphate incorporation into lipids under standard assay conditions. The glycerophosphate-dependent release of CoA correlated directly with incorporation of ^{14}C into the total lipid extract. The ratio of CoA to ^{14}C incorporation was approximately 1 at various concentrations of microsomes. The product of the reaction was identified as monoacylglycerophosphate by TLC in several solvent systems. Prior studies in mammals (7) and yeast (29) have suggested a similar product for *sn*-glycerol 3-phosphate esterification. However, other studies using homogenate or particulate preparations of liver have described phosphatidic acid and neutral glycerides as products of this esterification (30). It is likely that differences in microsomal preparation, the presence of albumin, or variability in the content of certain fatty acids in the incubation mixture (31) accounts for the variable products identified.

The role of serum proteins in activating GP-acyltransferase is not clear. Previous studies have also demonstrated this effect (32, 33). The activating effect is demonstrable regardless of assay technique, and is probably not caused by addition of metal ions or sulfhydryl groups.

Conversion of the long-chain acyl CoA to a more suitable substrate form by binding to protein, or binding of inhibitory substances, are possible explanations for the activation by proteins. The proteins studied had different binding capacities for palmitoyl CoA. However, this

binding was not correlated with the degree of GP-acyltransferase activation by these proteins. Thus, simple binding of palmitoyl CoA by protein is apparently insufficient for the activation of GP-acyltransferase.

Palmitoyl CoA hydrolysis releases palmitate, a product which inhibits GP-acyltransferase at high concentration. Albumin is known to bind palmitate, but the calculated amount of palmitate formed during the reaction is insufficient to cause inhibition of GP-acyltransferase even in the absence of albumin.

Although the mechanism and physiological importance of the activating effect of serum proteins on GP-transacylase must remain uncertain, the binding and removal of an inhibitory product of the reaction is a major possible explanation as yet unexcluded. The partial inhibition of GP-transacylase by diacylglycerophosphate suggests a possible role of glycerophosphatides in controlling the rate of GP-acyltransferase and offers a potential explanation of the albumin effect.

The activating effect of the serum protein fractions is of doubtful physiological significance since no such activator material was found in the particle-free cell supernate. This latter fraction and various protein preparations derived from it regularly inhibited GP-acyltransferase activity in vitro.

The effect of various potential inhibitors on GP-acyltransferase have been described. The inhibition by *N*-ethylmaleimide and DTN and the lack of inhibition by iodoacetamide substantiate the observations of Lands and Hart (7). These authors, using entirely different techniques, observed inhibition of hepatic acyltransferase activity by some sulfhydryl binding agents when *sn*-glycerol 3-phosphate was the substrate. No inhibition of the acylation of monoacylglycerophosphate occurred under the same conditions. This previous observation suggested the presence of separate enzymes mediating the acylation of these compounds. The data presented in this report strongly support this interpretation.

The physiological significance and specificity of the substances found to inhibit GP-acyltransferase in vitro, including diacylglycerophosphate, sodium palmitate, cytidine triphosphate, and metal ions, remains to be established.

Recent studies in bacteria have demonstrated that palmitoyl-ACP is an active substrate for *sn*-glycerol 3-phosphate acylation (34–37). The major product of this reaction is monoacylglycerophosphate (36). In some bacteria, palmitoyl CoA can be a substrate for transacylation (36), but in other strains palmitoyl CoA is not a direct substrate (37). The present study demonstrates that palmitoyl CoA is a likely substrate for GP-acyltransferase in mammalian liver and that no soluble protein fraction is required for this reaction. However, microsome-bound ACP or some similar protein may be required for *sn*-glycerol 3-phosphate acylation in mammals. The presence of a mammalian ACP has been suggested previously as part of the fatty acid synthetase complex (38). The present studies cannot exclude the participation of such a bound intermediate in the rat liver GP-acyltransferase reaction.

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