1-Acylglycerol 3-Phosphate Aeryltransferase from Rat Liver*

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SUMMARY

The substrate specificity of 1-acylglycerol 3-phosphate acyltransferase from rat liver microsomes for both acyl-CoAs and 1-acylglycerol 3-phosphate has been determined at levels below the critical micelle concentrations of the substrates. The fatty acid of the acylglycerol 3-phosphate is not important, but for the acyl-CoAs the specificity is oleyl > palmitoleyl > linoleyl > palmityl > myristyl > stearyl > lauryl.

1-Acylglycerol 3-phosphate acyltransferase appears to work only with monomeric substrates. The $K_m$ for palmitoyl-CoA is less than 0.1 $\mu M$, while the $K_m$ values for the 1-acylglycerol 3-phosphates are in the low micromolar range (5 to 25 $\mu M$), but are difficult to measure accurately because micelles form at 4 to 8 $\mu M$ 1-acylglycerol-3-P and the velocity ceases to increase. By contrast, acyl-CoA hydrolase appears to prefer micelles as substrate. This enzyme shows little activity at 3 $\mu M$ acyl-CoA, and shows 8 times more activity with palmitoyl-CoA than with any other acyl-CoA tested.

In order to plan the specificity studies, the critical micelle concentrations of the acyl-CoAs and 1-acylglycerol 3-phosphates used were determined by the dye adsorption technique with pinacyanol chloride. For acyl-CoA in 0.7 mM phosphate, 10 mM $K^+$, pH 6.9, the values were: lauryl, 9.5 $\mu M$; myristyl, 4 $\mu M$; palmitoyl, 3.6 (4 $\mu M$ in 50 mM Tris-HCl, pH 7.4); 6 $\mu M$ in deionized water; palmitoleyl, 2 $\mu M$; stearyl, 2 $\mu M$; oleyl, 4.5 $\mu M$; linoleyl, 5.5 $\mu M$. For 1-acylglycerol 3-phosphates in 50 mM Tris-HCl, pH 7.4, the values were: myristyl, 120 $\mu M$; palmitoyl, 35 $\mu M$; stearyl, 7 $\mu M$; oleyl, 23 $\mu M$; linoleyl, 34 $\mu M$; 1-acylglycerol 3-phosphate prepared from egg lecithin (80% palmitic), 37 $\mu M$.

In many naturally occurring phosphoglycerides unsaturated fatty acids are preferentially attached at position 2 and saturated fatty acids at position 1 (1). A likely origin for this asymmetric distribution of fatty acids would seem to be the esterification of n-glycerol 3-phosphate to form phosphatidic acid, since the biosynthesis of phosphatidic acid is the major source for the net synthesis of esterified fatty acids de novo in phosphoglycerides, and phosphatidic acid is a precursor of the other phosphoglycerides (2). Studies on the positional substrate specificity of n-glycerol 3-phosphate acyltransferase (EC 2.3.1.15) from guinea pig liver indicated that n-glycerol 3-phosphate was acylated in a nearly random manner (3); however, subsequent investigations with 1-acylglycerol-3-P acyltransferase from rat liver suggested that in this tissue unsaturated fatty acids were preferentially attached at position 2 of the synthesized phosphatidic acid (4). We have synthesized a number of synthetic 1-acylglycerol 3-phosphates, each containing a different fatty acid, and have conducted a more thorough investigation of 1-acylglycerol-3-P acyltransferase from rat liver by examining its substrate specificity as a function of the fatty acid composition of both the acyl donor, acyl coenzyme A, and the acyl acceptor, 1-acylglycerol-3-P. In addition, the critical micelle concentrations of the acyl CoA and 1 acylglycerol 3-phosphates used have been determined.

EXPERIMENTAL PROCEDURE

Preparation of Microsomes

Microsomes were prepared from the livers of male rats (Holtzman) weighing from 200 to 250 g. The livers were cut into small pieces, mixed with 0.25 M sucrose, 1 mM EDTA, and 0.3 mM dithiothreitol (40 ml per liver), and homogenized with a Potter-Elvehjem apparatus. Material sedimenting in 15 min at 15,000 $\times$ g was discarded, and the particles sedimenting in 90 min at 30,000 $\times$ g were suspended in 3 mM Tris-HCl, 1 mM EDTA, and 0.1 mM dithiothreitol, pH 7.4 (4 ml per liver), frozen quickly in an ethanol and Dry Ice mixture, and stored at $-20^\circ$ for up to, but not longer than, 1 week. Microsomal protein was determined by the biuret method of Gornall, Bardawill, and David (5), modified by the addition of 0.5 ml of 2% (w/v) sodium deoxycholate to all assay solutions.

Enzyme Assays

Enzyme activities were assayed at 30º by measuring the thiol groups of the CoA released with 4,4'-dipyridine disulfide (Aldrich), assuming a molar absorptivity of 19,800 at 324 nm (6). The change in absorbance was recorded continuously in 3-ml cuvettes (1-cm light path) with a 10-nv recorder attached to a Gilford optical density converter. Full scale deflections of 0.05, 0.1, and 0.5 absorbance unit were used and chart speed was adjusted to give slopes of approximately 45º. Microsomes were mixed with dipyridine disulfide at least 1 min prior to initiating the reaction by the addition of acyl-CoA. Microsomes contain both acyl-CoA hydrolase and 1-acylglycerol-3-P acyltransferase;
the former was determined in reaction mixtures with 1-acylglycerol-3-P omitted, and the latter from the difference in rates for reaction mixtures with and without 1-acylglycerol-3-P. At low acyl-CoA concentrations (less than 3 μM) hydrolyase activity is small compared with acyltransferase activity, and correction for hydrolyase activity was not always made.

Methods and Materials

Stearic, myristic, and linoleic acids and palmityl chloride were purchased from Calbiochem, oleic and palmitoleic acids from Mann, and lauric acid from Eastman. The fatty acids in the final acyl-CoA and 1-acylglycerol-3-P preparations were assayed by gas chromatography after conversion to the methyl esters by reaction with diazomethane. Retention times obtained for standards were used to identify peaks in the analyzed samples. The weight percentage for each peak was obtained by cutting the peak out of the recording paper, weighing it, and determining its percentage of the total weight for all the peaks in the sample. When solvents were removed from samples containing unsaturated fatty acids the rotary vacuum evaporator was thoroughly flushed with N₂ gas. Lipids containing unsaturated acids were not exposed to the air or left at room temperature longer than absolutely necessary and all preparations were stored at 0°C, or less, in a vacuum desiccator over P₂O₅ and KOH.

Ester groups were assayed by the hydroxamate method of Snyder and Stephens (7) with methyl palmitate (99.5%, Calbiochem) in methyl Cellosolve as a standard. Total phosphate was assayed by the method of Fiske and SubbaRow (8).

Phospholipid preparations were assayed by thin layer chromatography on Silica Gel G (Brinkmann Instruments Inc., Westbury, New York). Chloroform-methanol-7 N NH₄ (65:35:5) was used to check the purity of phosphatidylcholine (R_f = 0.4) and 1-acylglycerol-3-phosphorylcholine (R_f = 0.1), and to monitor column eluents containing these materials (9). Phosphatidic acid (R_f = 0.4) and 1-acylglycerol-3-P (R_f = 0.15) were separated with chloroform-methanol-30% methylamine (65:30:5) (10); plates developed in this system were placed in an oven at 60°C for 30 to 40 min before a pH-sensitive spray reagent was applied. To determine whether 1-acylglycerol-3-P (R_f = 0) was free of the potential contaminant, 1-acylglycerol-cyclich-3-phosphate (R_f = 0.5), plates were developed in chloroform-methanol-water (65:25:4) (11). Choline-containing phospholipids were located on the developed plates with the Dragendorf spray reagent (9). A pH-sensitive spray reagent containing 40 mg of bromothymol blue in 100 ml of 0.01 M KOH was routinely used to detect phosphatidic acid, 1-acylglycerol-3-P, and fatty acids, all of which gave a yellow spot against a blue background (10). Materials containing fatty acids were detected by exposing the plate to iodine vapors for 5 min (9).

Acyl-CoAs—Palmityl-CoA (K⁺ salt) was purchased from P-L Biochemicals, and stearyl-CoA (Grade II) from Sigma. Other acyl-CoAs, synthesized in 22 to 50% yield by acylating CoA (P-L Biochemicals) with the appropriate ethyl acyl carbonate (12), were purified by acid precipitation (13). Stearyl-CoA was also purified by acid precipitation. All of the acyl-CoAs migrated as single ultraviolet-absorbing spots (R_f = 0.0 to 0.8) on Whatman No. 1 paper with the solvent 1-butanol-acetic acid-water (5:2:3). In 3 mM Tris-HCl, pH 7.4, the A₄₅₀ : A₄₃₀ ratio was 0.66 or 0.67 for all preparations. Acyl-CoA concentrations were determined spectrophotometrically, assuming a molar absorptivity of 15,400 at 260 μM (14). The purity of the acyl-CoAs was checked by gas chromatography of the fatty acids liberated by hydrolysis in base. Oleyl, myristyl, stearyl, and palmityl-CoA were >98% pure. The other acyl-CoAs were lauryl-CoA, 12:0, 92%; 14:0, 8%; linoleyl-CoA, 12:0, 2%, 14:0, 8%, 18:2, 88%, unidentified, 2%; palmitoleyl-CoA, 12:0, 3%, 14:0, 6%, 16:1, 89%, unidentified, 2%.

1-Acylglycerol 3-Phosphates—The first step in the preparation of 1-acylglycerol 3-phosphates of known composition was the extraction of phosphatidylcholine from the yolks of chicken eggs according to the method of Singleton et al. (15). Phosphatidylcholine was subsequently decylated by reaction with hydroxylamine and the glycerol 3-phosphorylcholine formed was precipitated from ethanol as the cadmium chloride complex (16). This complex was then acylated with the appropriate acyl chloride under anhydrous conditions (17). After reaction, the material insoluble in the chloroform was extracted by washing three times with water (7%, v/v). After rotary evaporation of the chloroform layer, the residue was dissolved in 5 ml of chloroform and added to a column (2 x 12 cm) of Bio-Sil HA (Bio-Rad Laboratories, Richmond, California). Fatty acids were eluted with 100 ml of chloroform, phosphatidylcholine with 100 ml of chloroform-methanol (3:2), and lysophosphatidylcholine with 100 ml of methanol. Yields of phosphatidylcholine ranged from 10 to 35%, based on the quantity of glycerol 3-phosphorylcholine initially present.

Phosphatidylcholine was converted to 1-acylglycerol 3-phosphorylcholine by reaction with phospholipase A from Crataegus adansoniensis (Ross Allen Reptile Gardens) (18) which is specific for cleavage of the ester bond at position 2 (19). When preparing the palmityl and stearyl compounds, chloroform (2%, v/v) was added to enhance the solubility of the synthetic phosphatidylcholine, and the reaction time was extended to 20 hours. This reaction was also carried out with phosphatidylcholine isolated from egg yolks, so that after the subsequent cleavage with phospholipase D (see below) the final 1-acylglycerol-3-P contained the fatty acids present in the original lecithin (80% palmitic, 16% stearic). This material was used in all studies except where the substrate specificity for 1-acylglycerol-3-P was being investigated.

1-Acylglycerol-3-P was prepared from 1-acylglycerol 3-phosphorylcholine by the action of cabbage phospholipase D (Calbiochem) (11, 20). When preparing the palmityl and stearyl compounds, ether (20%, v/v) was added to aid in the solubilization of the 1-acylglycerol 3-phosphorylcholine, and the reaction was extended to 20 hours. A small amount of salt in the final product was removed by extraction of an ether solution with an equal volume of 0.3 M HCl, followed by water. After removal of ether, the product was stored as the free acid over P₂O₅ in vacuum, and prior to use was dissolved in 3 mm Tris-CI, pH 7.4. Esterified fatty acid to phosphate ratios for the products varied from 0.06 to 1.20. All products gave only one spot on thin layer chromatograms with two different solvent systems (see above). The fatty acid compositions of these products determined by gas chromatography were the same as for the corresponding acyl-CoAs (see above).

Determination of Critical Micelle Concentrations—The critical micelle concentrations of acyl-CoAs were determined by the dye adsorption technique described previously (21). When the 1-acylglycerol 3-phosphates were examined, 1-cm cuvettes holding a total volume of 3 ml were used, and solutions were prepared by mixing 1.0 ml of 0.15 M Tris-HCl, pH 7.4, 1.0 ml of
15 μM pinacyanol chloride (Eastman), an aliquot of 1-acylglycerol-3-P (dissolved in 3 mM Tris-HCl, pH 7.4), and water to 3.0 ml. The absorbance at 610 μm was determined versus a blank of 1.0 ml of 0.15 M Tris-HCl, pH 7.4, and 2.0 ml of water.

RESULTS

Critical Micelle Concentrations of Substrates—Both of the substrates of 1-acylglycerol-3-P acyltransferase possess the type of structure common to surfactants; namely, a nonpolar hydrophobic group and a polar hydrophilic group. Aqueous solutions of such molecules show reversible association of a large number of monomers into micelles of colloidal size above a characteristic concentration called the critical micelle concentration (22, 23). Since there is considerable evidence that in some cases, the free monomer (24), and in other cases, the micelle, is the substrate for a given enzyme (25), it is important to know the critical micelle concentrations of the substrates in order properly to interpret the observed kinetics and to plan experiments designed to determine substrate specificity.

By light scattering and dye adsorption techniques the critical micelle concentration of palmitoyl-CoA has previously been determined to be 2 to 4 μM in 6.7 mM potassium phosphate, pH 6.9 (21). Critical micelle concentrations of the other acyl-CoAs used in the present work were determined by the dye adsorption technique in the same phosphate buffer, and the values are shown in Table I. No increase in the absorbance by 610 μm was noted for concentrations of decyl-CoA up to 58 μM; thus if micelles form, they do so at a higher concentration. The critical micelle concentrations of the different 1-acylglycerol 3-phosphates were determined by the dye adsorption technique in 50 mM Tris-HCl, pH 7.4, and the values are given in Table I. Fig. 1 shows representative curves for stearyl, oleyl, and linoleyl-glycerol 3-phosphates.

In studies of 1-acylglycerol-3-P acyltransferase both acyl-CoA and 1-acylglycerol-3-P are present in the reaction mixture; thus if mixed micelles can form, the apparent critical micelle concentration may be different from the critical micelle concentrations of either substrate. To check this point, the apparent critical micelle concentration was determined by the dye adsorption technique in 50 mM Tris-HCl, pH 7.4, for a solution containing a 1:2 ratio of palmitoyl-CoA and 1-acylglycerol-3-P (derived from egg lecithin). The critical micelle concentration of this system was 8 μM total surfactant concentration. This is consistent with the theory of binary surfactant systems, which predicts that the critical micelle concentration of a mixture will fall between the critical micelle concentrations of the two components (26).

Calculation of the actual concentrations of free substrates present in reaction mixtures is also complicated by nonspecific binding, particularly by acyl-CoA, to the microsomes. Palmitoyl-CoA causes substrate inhibition of several enzymes (4, 27), presumably by some type of protein-surfactant interaction. The addition of heat-treated microsomes to systems inhibited by high (50 to 100 μM) concentrations of palmitoyl-CoA resulted in increased enzymatic activity which was attributed by Lands and Hart (4) to the binding of inhibitory acyl-CoA by the added microsomes. That such binding also takes place at the concentrations of acyl-CoA employed in the present studies was shown by measuring the velocity of acyl-CoA hydrolase with and without added heat-treated microsomes or bovine serum albumin (Fig. 2). These inhibitions by bovine serum albumin and heat-treated microsomes indicate that the substrate concentration had been decreased in each case by the binding phenomenon; similar inhibition occurs in the presence of β-Schardinger dextrin, which specifically combines with molecules containing adenine (21, 28). With 18 μM palmitoyl-CoA, 3.3 mM dextrin gave 70% inhibition of hydrolase activity.

Basic Enzyme Properties—So that initial velocities could be measured as accurately as possible, the concentrations of 1-acylglycerol-3-P and palmitoyl-CoA in initial studies with 1-acylglycerol-3-P acyltransferase were set at 6 μM and 3 μM. Although the estimated critical micelle concentration for such a system was 8 μM total surfactant, the microsomes would be expected to decrease the palmitoyl-CoA concentration by nonspecific binding so that micelles would not form to any extent in the reaction mixture.

Table I

Critical micelle concentrations of acyl-CoAs and 1-acylglycerol 3-phosphates determined by dye adsorption

The experimental procedure is described under "Methods and Materials."

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Acyl-CoA</th>
<th>1-Acylglycerol-3-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>Lauryl</td>
<td>9.5</td>
<td>120</td>
</tr>
<tr>
<td>Myristyl</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Palmityl</td>
<td>3.6, 4, 6</td>
<td>35</td>
</tr>
<tr>
<td>Palmitoleyl</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Stearyl</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Oleyl</td>
<td>4.5</td>
<td>23</td>
</tr>
<tr>
<td>Linoleyl</td>
<td>5.5</td>
<td>34</td>
</tr>
<tr>
<td>&quot;Egg lecithin&quot;</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

* In 6.7 mM phosphate, 10 mM K+, pH 6.9.
α In 50 mM Tris-HCl, pH 7.4.
β Reference 21.
γ In deionized water.

*1-Acylglycerol-3-P was prepared from egg phosphatidylcholine by hydrolysis with phospholipases A and D (see "Methods"). The composition of the final product determined by gas chromatography was palmitic (80%), stearic (16%), and oleic acid (2%), with traces of lauric, myristic, and linoleic acids.

![Fig. 1. Determination of the critical micelle concentrations of 1-acylglycerol 3-phosphates.](http://www.jbc.org/)

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*Fig. 1. Determination of the critical micelle concentrations of 1-acylglycerol 3-phosphates. , stearyl; , oleyl; , linoleyl. See "Methods and Materials" for experimental details.*
The pH optimum for 1-acylglycerol-3-P acyltransferase was 7.8 to 8.2; a similar value has been reported for other acyltransferases (29). However, subsequent experiments were conducted at pH 7.4 in order to facilitate comparison with the majority of results published on acyltransferases. At pH 7.4, the velocity was 80 to 85% of that measured at pH 7.8 to 8.2.

As shown in Fig. 3, the initial velocity of 1-acylglycerol-3-P acyltransferase varied with the buffer concentration, although the velocity of acyl-CoA hydrolase did not. With 50 mM Tris-HCl, the reaction rate was 170% of that observed at 5 mM. A buffer concentration of 50 mM was chosen for subsequent studies.

The velocity of the acyltransferase reaction was a linear function of enzyme up to 0.3 mg of protein per 3 ml (Fig. 4). The observed linear relationship suggests that the apparent $K_m$ of 1-acylglycerol-3-P acyltransferase for palmitoyl-CoA is less than 0.3 mM, since the palmitoyl-CoA concentration was apparently still sufficient to saturate the enzyme, although the original concentration (3 mM) should have been decreased by nonspecific binding. The inhibition observed at protein concentrations greater than 0.3 mg per ml suggests that at these protein levels the palmitoyl-CoA concentration is depleted below a saturating level. A pattern similar to that shown in Fig. 4 has been observed with dihydroxyacetone phosphate acyltransferase (30).

When the concentration of palmitoyl-CoA was varied in an effort to determine a Michaelis constant, reactions were run with and without 6 μM 1-acylglycerol-3-P (derived from egg lecithin), since microsomes contain acyl-CoA hydrolase as well as 1-acylglycerol-3-P acyltransferase (4). The results in Fig. 5 show that the hydrolase is relatively inactive at low palmitoyl-CoA levels, but is responsible for most of the reaction at high levels (this is a unique property of palmitoyl-CoA; other acyl-CoAs are not nearly so active with the hydrolase (see Fig. 9)). In contrast, net acyltransferase activity is constant from 1 to 3 μM, and accounts for most of the palmitoyl-CoA split; it declines at very high palmitoyl-CoA concentrations.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Inhibition of acyl-CoA hydrolase by added protein. Reaction mixtures contained 50 mM Tris-HCl, pH 7.4, 300 μM 4,4'-dipyridine disulfide, palmitoyl-CoA as indicated, and 0.033 mg per ml of microsomal protein with a total volume of 3.0 ml. ●, no addition; ○, plus 0.1 mg per ml of bovine serum albumin; ▲, plus 0.1 mg per ml of heat-treated microsomes (heated at 80–85°C for 12 min at a concentration of 3 per mg ml, which destroys hydrolase activity).

**Fig. 4.** Activity versus enzyme curve for acyltransferase activity. Reaction mixtures were similar to those in Fig. 2 with 3 μM palmitoyl-CoA, 6 μM 1-acylglycerol 3-P from egg lecithin, 1 mM EDTA added, and microsomal protein varied as indicated. Hydrolase activity measured in the absence of 1-acylglycerol-3-P has been subtracted from each value.

**Fig. 5.** Acyltransferase and hydrolase activities as a function of palmitoyl-CoA concentration. Reaction mixtures were similar to those in Fig. 4 except that microsomal protein was 0.033 mg per ml and palmitoyl-CoA was varied as indicated. ▲, hydrolase activity (1-acylglycerol-3-P omitted); ○, acyltransferase plus hydrolase activities; ●, net acyltransferase activity.
FIG. 6. Reciprocal plot of acyltransferase plus hydrolase activities. Data are from Fig. 5.

Fig. 7. Acyltransferase activity as a function of 1-acylglycerol-3-P concentration. Reaction mixtures were similar to those in Fig. 3, except that palmityl-CoA was 2.5 µM, and 1-acylglycerol-3-P from egg lecithin was varied as indicated. A, velocity versus 1-acylglycerol-3-P concentration; B, reciprocal plot of the same data.

From these data one can conclude that the $K_m$ of palmityl-CoA for the acyltransferase is less than 0.1 µM, and that only free monomeric palmityl-CoA molecules act as substrates. By contrast, the hydrolase seems to prefer the micellar form, and the initial velocity of hydrolysis continues to increase up to 45 µM palmityl-CoA (data not shown), above which it plateaus. The presence of the two enzyme activities is very clearly shown by Fig. 6, which is a reciprocal plot of the total initial velocity in the presence of both substrates.

When the velocity of the acyltransferase was examined as a function of the 1-acylglycerol-3-P concentration (Fig. 7), the effects of micelle formation were clearly seen. The velocity was constant from 9 to 20 µM 1-acylglycerol-3-P, although the apparent $K_m$ defined by the lower 1 acylglycerol-3-P concentrations (Fig. 7B) appears to be around 20 µM (it cannot be accurately determined because of the long extrapolation required and the difficulty in determining the initial velocity at low substrate levels). These data are consistent with an apparent critical micelle concentration of 9 µM 1-acylglycerol-3-P (11.5 µM total surfactant), and suggest that only the free monomers are substrates. The inhibition at higher 1-acylglycerol-3-P concentrations is presumably caused either by protein denaturation or by a rapidly diminishing monomer concentration of palmityl-CoA as the result of its incorporation into mixed micelles, or both.

Substrate Specificity—The specificity of 1-acylglycerol-3-P acyltransferase for the fatty acid composition of both acyl-CoA and 1-acylglycerol-3-P was investigated in a series of comparative kinetic studies. In these experiments, groups of data to be directly compared were, as far as possible, obtained on the same day, with the same set of reagents, and with the same preparation of microsomes (the specific activity of different microsomal

Fig. 8. Kinetics of acyltransferase with different acyl-CoAs. Reaction mixtures were similar to those in Fig. 5, and acyl-CoA concentrations were varied as indicated. \( \nabla \), oleyl; \( \bullet \), palmityl; \( \cdot \), myristyl; \( \downarrow \), stearyl; \( \circ \), lauryl. These velocities represent combined acyltransferase and hydrolase activities, but hydrolase activities are negligible below 3 µM (see Fig. 9).

Fig. 9. Kinetics of hydrolase with different acyl-CoAs. Reaction mixtures were similar to those in Fig. 8, except that 1-acylglycerol-3-P was omitted. Symbols are the same as those in Fig. 8.
tion of acyl-CoA. 1-acylglycerol-3-P acyltransferase transferred
without 6
erol-3-P acyltransferase and acyl-CoA hydrolase, although the
rates in each case were slightly slower than with oleyl-CoA.
was negligible. In experiments not shown, palmitoleyl-CoA
at the lower substrate concentrations where hydrolase activity
able selectivity for t'he fatty acid composition of the acyl-CoA

In contrast to the specificity shown for the fatty acid composi-
tions of the acyl-CoAs determined in the present work, suggesting
preparations varied significantly). In the first series of experi-
ments the specificity for acyl-CoA was determined with and
without 6 μM oleyl-CoA derived from egg phosphatidycholine
(Figs. 8 and 0). The acyltransferase (Fig. 8) showed consid-
erable selectivity for the fatty acid composition of the acyl-CoA
at the lower substrate concentrations where hydrolase activity
was negligible. In experiments not shown, palmitoleoyl-CoA
and linoieryl-CoA gave nearly identical patterns with 1-acylgly-
colein 3-phosphate acyltransferase and acyl-CoA hydrolase, although the
rates in each case were slightly slower than with oleyl-CoA.
In contrast to the specificity shown for the fatty acid composi-
tion of acyl-CoA, 1-acylglycerol-3-P acyltransferase transferred
oleic acid to 1-acylglycerol-3-P at nearly the same rate for all
synthetic 1-acylglycerol 3-phosphates tested (Fig. 10). The
substrate inhibition observed with 1-stearylglycerol-3-P at
concentrations greater than 9 μM is consistent with the very low
critical micelle concentration of 1-stearylglycerol-3-P.
The velocity of the acyltransferase was then measured in the
presence of all the substrate combinations possible with the
available acyl-CoAs and 1-acylglycerol 3-phosphates. A 0 μM
concentration of 1-acylglycerol-3-P was chosen since all of the
synthetic 1-acylglycerol 3-phosphates gave essentially their
maximum velocity at this concentration, yet it was below the
level of substrate inhibition for 1-stearylglycerol-3-P. The
acyl-CoA concentration was set at 2.5 μM so that the contribu-
tion of acyl-CoA hydrolase to the measured velocity would be
insignificant. The results shown in Table II indicate that 1-acyl-
glycerol-3-P acyltransferase is specific for the fatty acid of the
acyl-CoA, but is not significantly specific for the fatty acid in
the 1-acylglycerol-3-P.

**DISCUSSION**

In the present studies the critical micelle concentration was
defined as the concentration at which the absorbance of pinaeyla-
nol chloride at 610 mg began to increase. In physical terms this
marks the concentration at which the dye is first incorporated
into micelles, and critical micelle concentrations defined in this
manner are generally lower than critical micelle concentrations
measured by other techniques because the dye is capable of in-
ducing the formation of micelles (31). Since the mole fraction
of pinacyanol chloride in the surfactant-indicator system was at
least 0.04 (a relatively high value) at the estimated critical micelle
concentration, the values obtained in the present studies may be
somewhat lower than those determined by a different method.
For palmitoyl-CoA, however, both light scattering and dye binding
studies have indicated that the critical micelle concentration was
about 2 to 4 μM in the presence of 10 mM K+ at pH 6.9 (21), so
that the values determined in the present work are probably
valid.

By surface tension measurements, Robinson and Saunders
determined the critical micelle concentration of 1-acylglycerol
3-phosphorylcholine derived from egg phosphatidylcholine to be
22 μM at 25° without added salt (29). In the present study
1-acylglycerol-3-P prepared from egg phosphatidylcholine had a
critical micelle concentration of 37 μM at 25° with 50 mM Tris-
HCl, pH 7.4. These values are reasonable since a surfactant
with a net negative charge should have a higher critical micelle
concentration than an analogous molecule with a swittherionic
group. The critical micelle concentrations of the other 1-acyl-
glycerol-3-P preparations varied according to chain length and
degree of unsaturation in a manner consistent with observations
made on similar systems (33).

Based on the evidence in the literature, acyl-CoA apparently
interacts with a number of different enzymatic proteins. Wie-
land, Welsch, and Eger-Neufeldt (34) have reported that citrate
synthase was 50% inhibited at the concentrations listed for the
following acyl-CoAs: lauryl-CoA, 99 μM; palmitoyl-CoA, 4.2 μM;
oleyl-CoA, 9.6 μM; stearoyl-CoA, 3 μM. Acetyl-CoA carboxylase
was also inhibited and $K_i$ values for various acyl-CoAs were:
lauryl-CoA, 74 μM; myristoyl-CoA, 11 μM; palmitoyl-CoA, 7.2
μM; oleyl-CoA, 1.3 μM; stearoyl-CoA, 0.7 μM (35). These inhibi-
tion data correlate quite well with the critical micelle concen-
trations of the acyl-CoAs determined in the present work, suggesting

![Fig. 10. Kinetics of acyltransferase with different 1-acylglyc-
erol 3-phosphates. Reaction mixtures were similar to those in
Fig. 3, except that 2.5 μM oleyl-CoA was the acyl-CoA and 1-acyl-
glycerol-3-P concentrations were varied as indicated. A: O, palmitoyl; V, stearyl; B: myristyl. O, oleyl; O, linoieryl. The
velocities are not corrected for hydrolase activity.](image-url)
that the observed inhibitions are largely due to the surfactant properties of the acyl-CoAs.

Garland, Shepherd, and Yates (30) have reported that microsomes isolated from rat liver contain 0.1 mumole of bound long chain acyl-CoA per mg of protein. The monomer concentration in equilibrium with this bound material would presumably not be very large; indeed, no acyl-CoA was detected in the supernatant when the microsomes were isolated, and the assay was sensitive to concentrations of at least 0.1 μM (36). These observations suggest that the concentration of unbound acyl-CoA in vivo is probably less than 0.1 μM. For 1-acylglycerol-3-P acyltransferase, the apparent Kₐ for palmitoyl-CoA of less than 0.1 μM observed in the present studies is consistent with the low level in vivo, since the apparent Kₐ values of enzymes generally reflect the concentrations in vivo of their substrates (37). The higher Kₐ values for acyl-CoAs frequently reported in the literature reflect the failure to measure initial velocities below critical micelle concentrations; the way in which such artifacts occur has been explained by Zahler and Cleland (24).

The investigations of the substrate specificity of 1-acylglycerol-3-P acyltransferase revealed that the fatty acid composition of acyl-CoA affects the reaction rate to a greater extent than does the fatty acid composition of 1-acylglycerol-3-P. Fig. 8 shows that the maximum velocity was closely a function of the fatty acid composition of acyl-CoA, whereas the maximum velocities in the absence of 2.5 μM oleoyl-CoA did not vary appreciably with the fatty acid composition of 1-acylglycerol-3-P (Fig. 10). Although the data in Fig. 10 show that the Kₐ values for the various 1-acylglycerol-3-phosphates are in the micromolar range (5 to 25 μM), precise values cannot be obtained because the velocity drops increasing when micelles form and the data at low substrate levels are not numerous or precise enough. In Table II, the velocities in the horizontal rows, which reflect the specificity for 1-acylglycerol-3-P, vary significantly less than the velocities in the vertical rows, which reflect the specificity for acyl-CoA. A similar conclusion has been reached by two different groups who studied the substrate specificity of 1-acylglycerol-3-phosphorylcholine acyltransferase (38, 39). Based on the data in Table II, 1-acylglycerol-3-P acyltransferase shows the following specificity for acyl-CoA: oleoyl-CoA > palmitoyl-CoA > linoleoyl-CoA ≈ palmitoyl-CoA > myristoyl-CoA > stearoyl-CoA > lauroyl-CoA. These results indicate that unsaturated fatty acids are the preferred substrates for transfer to position 2 of 1-acylglycerol-3-P.

Hill and Lands (40) recently reported the use of 56 μM 1-oleoyl-3-P as the acyl acceptor for a variety of acyl-CoAs (at 19 μM). With rat liver microsomes they obtained a relative distribution of velocities very similar to the more detailed results reported here. These observations contrast sharply with the results of Stoffel, De Tomas, and Schieffer (41), who have reported that the rat liver enzyme showed no specificity for the fatty acid component of acyl-CoA.

Recently, Hill, Husbands, and Lands (42) noted that tissue slices of rat liver incorporated unsaturated fatty acids into position 2 of phosphatidic acid almost exclusively; also, in phosphatidylethanolamine isolated from rat liver, nearly all of the fatty acids at position 2 were unsaturated (43). Therefore, the positional specificity exerted in tissue in vivo and in tissue slices must be more pronounced than that observed with the microsomal system examined in the present studies which transferred palmitic acid to 1-acylglycerol-3-P at a high rate.

While phosphatidic acid synthesis from glycerol-3-P may involve 1-acylglycerol-3-P as an intermediate, there has been no direct demonstration that 1-acylglycerol-3-P acyltransferase plays an essential role in the synthesis of phosphatidic acid from glycerol-3-P in liver. However, Hajra (30) has reported that dihydroxyacetone phosphate was acylated by liver tissues, and that the acyl oxydihydroxyacetone phosphate so formed could be reduced to 1-acylglycerol-3-P (44). In mitochondria the acyltransferase esterifying dihydroxyacetone phosphate showed a preference for C-15, C-16, and C-17 saturated fatty acids, but the microsomal enzyme utilized unsaturated fatty acids nearly as well as saturated acids (30). Although the net contribution of this pathway to glycerolipid synthesis has not been evaluated, it may be a significant source of 1-acylglycerol-3-P containing chiefly saturated fatty acids.

Acyl-CoA hydrolase, which is a part of pigeon liver fatty acid synthase, has recently been shown to be highly specific for acyl-CoA containing C-16 or C-18 fatty acids (45). In the present studies the rat liver enzyme showed a markedly faster reaction rate with palmitoyl-CoA than with any of the other acyl-CoAs tested, but the physiological importance of this enzyme is not known.

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