The Effect of Glycerol 3-Phosphate on Fatty Acid Synthesis*

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(Received for publication, June 1, 1965)

The synthesis de novo of fatty acids by particle-free cell extracts is inhibited by long chain fatty acyl coenzyme A derivatives such as palmitoyl coenzyme A (1-5). On the other hand, fatty acid synthesis is stimulated by the addition of microsomes (6-10). The enzymes that transfer long chain fatty acyl groups from coenzyme A to glycerol 3-phosphate and its derivatives are also located in the microsomes (10-14). These observations suggest that the microsomal stimulation of fatty acid synthesis may be caused at least in part by transfer of acyl groups from long chain fatty acyl coenzyme A to glycerol 3-phosphate. The present report describes the effect of glycerol 3-phosphate on the synthesis of fatty acids. A preliminary account of this work has appeared (15).

EXPERIMENTAL PROCEDURE

Animals—Rats were obtained from Charles River Breeding Laboratories and Holtzman. The animals were maintained on a stock ration of Wayne Lab-Blox. Rats that had been deprived of food for 2 days were made diabetic by intraperitoneal injection of a freshly prepared solution of alloxan monohydrate at a dosage of 100 mg per kg. Rats were judged to be diabetic if their urinary output exceeded 40 ml per day, and if they excreted more than 3 g of glucose (range 3 to 13 g) per day 5 to 7 days after injection with alloxan. Obese mice and their normal, nonobese siblings (Strain C57 BL-6J-ob) were obtained from The Jackson Laboratory.

Materials and Methods—Palmitic acid-9,10-3H and citric acid-1,5-4C were purchased from New England Nuclear. DL-Glycerol 3-phosphate was obtained from Sigma. Enzymatic assay (16) showed that it contained 46% of L-glycerol 3-phosphate. Palmitoyl-CoA was prepared by the method of Kornberg and Pricer (10), and was purchased from Sigma. Lipids were separated according to the method of Borgström (17). Other methods were as described previously (9, 18).

RESULTS

Reaction mixtures used in studies of fatty acid synthesis usually have not contained glycerol 3-phosphate, because this substance was not considered to affect fatty acid synthesis. Fig. 1 shows that the rate of fatty acid synthesis is about 5 times greater in the presence than in the absence of glycerol 3-phosphate. In seven similar experiments, the range for the stimulation of fatty acid synthesis was 3.2 to 8.4-fold, and the average was 5-fold.

* This work was supported by the Medical Foundation, Boston, Massachusetts, and the National Science Foundation (G-14614 and GB-833). C. Howard was supported by a training grant from the National Institute of Neurological Diseases and Blindness (STINB5241-06). This is Publication No. 371 of the Graduate Department of Biochemistry, Brandeis University.
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FIG. 1. Effect of glycerol 3-phosphate on citrate incorporation into fatty acids. High speed supernatant (HSS throughout figures) and microsomes were prepared from the liver of a rat that was deprived of food for 48 hours and refed with white bread for 62 hours. The reaction mixture contained 20 mM citrate-1,5-'*C (8.0 X 10⁶ c.p.m. per μmole), 50 mM glycylglycine buffer, 10 mM MgCl₂, 0.5 mM MnCl₂, 30 mM glutathione, 0.26 mM CoA, 0.68 mM TPN, 5 mM glucose 6-phosphate, 5 mM ATP, 25 mM NaHCO₃, 15 mM creatine phosphate, 10 μg of creatine phosphokinase, approximately 0.1 M sucrose, high speed supernatant, (2.0 mg of protein), and microsomes. The reaction was started by the addition of high speed supernatant and microsomes. The pH was 7.5 to 7.6. Incubations were in a final volume of 1.0 ml, at 38°, for 30 min. In A, the amount of glycerol 3-phosphate was varied. The amounts of microsomal protein added were: ●, 1.2 mg; and ▲, 0.6 mg. In B, the amount of microsomal protein was varied. The concentrations of D, L-glycerol 3-phosphate were (mM): ●, 8.9; ■, 4.45; ▲, 1.78; ▼, 0.89; and □, 0.

FIG. 2. Effect of microsomes prepared from rats deprived of food on fatty acid synthesis in the presence (●, ■, ▲, ●, ▲) and absence (○, ▼, △, ○, △) of glycerol 3-phosphate. The high speed supernatant used for all points shown was prepared from liver of a rat that had been deprived of food for 63 hours and refed a diet high in glucose (19) for 48 hours. Liver microsomes were prepared from the same rat (●), or from rats that had been deprived of food for 24 hours (▲), 48 hours (▲), 72 hours (■), and 96 hours (●). For other conditions, see Fig. 1.

which is much less than the maximum stimulation observed with liver preparations (Figs. 1 to 4). A possible reason for this lower degree of stimulation may be found in the shorter chain lengths of the fatty acids synthesized by lactating mammary gland as compared to liver. Fatty acyl thioesters of CoA inhibit fatty acid synthesis, the degree of inhibition increasing sharply with the chain length (4, 5). On the basis of published Kᵢ values (4), and for a given amount of precursor incorporation, end product inhibition would be expected to be less in mammary gland than in liver. Release of end product inhibition by glycerol 3-phosphate would therefore lead to a relatively smaller degree of stimulation.

The results presented so far show that the rate-limiting step of fatty acid synthesis is in the high speed supernatant, and not in the microsomes. Different nutritional states or diabetes do not appear to affect the capacity of microsomes to stimulate fatty acid synthesis in the presence of glycerol 3-phosphate (Figs. 2 to 4). Earlier reports indicated that this capacity varies widely (6, 22, 23), but this may have been a reflection of small but different amounts of glycerol 3-phosphate, diglycerides, and other acceptors of long chain acyl groups in the microsomes. The microsomes themselves contain some fatty acid-synthesizing activity (24), but this is far too low to account for the large stimulation observed when microsomes are added to the enzymes of the high speed supernatant.

The results do not show which particular step of fatty acid synthesis is accelerated by glycerol 3-phosphate and microsomes. We have tested the hypothesis that glycerol 3-phosphate stimulates fatty acid synthesis because it releases an inhibition caused by end-products. The rate of fatty acid synthesis in the presence of glycerol 3-phosphate and microsomes became slower the later these constituents were added after starting the reaction (Fig. 6).
FIG. 3. Effect of glycerol 3-phosphate on fatty acid synthesis by preparations of livers from starved and refed rats, and from diabetic rats. For conditions, see Fig. 1. A, •, ▲, presence; and △, ○, ▼, absence of 9.5 mM n-l-glycerol 3-phosphate. The high speed supernatants used in this experiment were prepared from livers of rats in three different metabolic states, namely, a rat deprived of food for 2 days and then refed a diet high in glucose for 2 days (left-hand panel); a diabetic rat fed a diet high in fructose for 2 days (right-hand panel), and a diabetic rat maintained on laboratory chow (right-hand panel, ---). In addition, the reaction mixtures contained liver microsomes prepared from the following sources: •, the food-deprived and refed rat; ▲, the diabetic rat fed diet high in fructose; and △, the diabetic rat maintained on chow. All results obtained with the high speed supernatant prepared from the liver of the diabetic rat maintained on chow fell below --- (right-hand panel). Citrate incorporation by this high speed supernatant alone was 0.4 micromoles per mg of protein per hour. Addition of glycerol 3-phosphate and microsomes prepared from the liver of the starved and refed rat increased this rate to 2.5 micromoles per mg of high speed supernatant protein per hour.

However, the unstimulated rate dropped off more rapidly than the stimulated rate. Therefore, the degree of stimulation actually increased, the later glycerol 3-phosphate and microsomes were added. When glycerol 3-phosphate and microsomes were added 0, 16, 31, and 60 min after starting the incubation, the rate of citrate incorporation into fatty acids was 3.3, 3.9, 12, and 7.6 times greater, respectively, than the corresponding rates observed in the absence of glycerol 3-phosphate.

Fatty acid synthesis was inhibited by addition of palmityl-CoA to the reaction mixture. The higher the concentration of palmityl-CoA that was added, the less were glycerol 3-phosphate and microsomes able to reverse the inhibition by palmityl-CoA. Nevertheless, for a given concentration of palmityl-CoA, the rate of fatty acid synthesis in the presence of glycerol 3-phosphate and microsomes was considerably greater than the rate obtained with high speed supernatant alone (Fig. 7).

Analyses of the radioactive end products showed that addition of microsomes, or of glycerol 3-phosphate plus microsomes, led to a shift of 14C incorporation from neutral lipids to free fatty acids. This may have been due to the removal of endogenous acceptors by esterification with the added, unlabeled palmityl-CoA. When high speed supernatant, microsomes, and glycerol 3-phosphate were present, addition of 60 mm of palmityl-CoA led to a shift of "C incorporation from neutral lipids to free fatty acids. This may have been due to the removal of endogenous acceptors by esterification with the added, unlabeled palmityl-CoA. When high speed supernatant, microsomes, and glycerol 3-phosphate were present, addition of 60

FIG. 4. Effect of glycerol 3-phosphate on citrate incorporation into fatty acids by preparations from obese mice and from their nonobese siblings. High speed supernatants were prepared by pooling three livers each of mice that were obese (right-hand panel) and nonobese (left-hand panel). In addition, the reaction mixtures contained liver microsomes prepared from: •, obese mice; ▲, nonobese siblings. For conditions, see Fig. 1. A, •, presence; and △, ○, absence of 9.5 mM n-l-glycerol 3-phosphate.

FIG. 5. Effect of glycerol 3-phosphate on fatty acid synthesis by preparations of a lactating mammary gland of rat. For conditions, see Fig. 1. •, presence; and △, ○, absence of 9.5 mM n-l-glycerol 3-phosphate. The protein fractions were prepared from a rat with a litter of 8 that had been lactating for 15 days.
μM palmityl-CoA had very little effect on the pattern of the radioactive products, although fatty acid synthesis was inhibited by 66%. These results suggest that in the absence of glycerol 3-phosphate and microsomes, long chain acyl-CoA is hydrolyzed to free fatty acids and CoA, whereas in the presence of glycerol 3-phosphate and microsomes the long chain acyl group is transferred directly from CoA to glycerol 3-phosphate. This interpretation is borne out by a separate experiment in which 62.5 μM 3H-palmityl-CoA was added to the reaction mixture (Table II). In the absence of glycerol 3-phosphate and microsomes, most of the palmityl-CoA was converted to free palmitate, whereas in their presence, it was converted to neutral and phospholipids. Phosphatidic acid phosphatase occurs in the "intermediate" cell fraction, which possesses sedimentation characteristics similar to the lysosomes (14). The relative amounts of neutral and phospholipids formed in the experiments shown in Tables I and II may be a function of the amount of this cell fraction present in the microsomal fraction.

**Table I**

<table>
<thead>
<tr>
<th>Products of citrate incorporation in presence and absence of glycerol 3-phosphate and microsomes</th>
</tr>
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<tbody>
<tr>
<td>The rats were fasted for 2 days and refed with a diet high in glucose for 3 days. The cell fractions were prepared from pooled livers of two animals. Reaction conditions were as described in Fig. 1, and all tubes contained 2 mg of high speed supernatant protein. Where indicated, the reaction mixture also contained microsomes (1.0 mg of protein) and 9.2 mM D-glycerol 3-phosphate. The experiment was run in (A) the absence, and (B) the presence of 90 μM palmityl-CoA.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Additions</th>
<th>Citrate incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>A. None</td>
<td>36</td>
</tr>
<tr>
<td>Microsomes</td>
<td>50</td>
</tr>
<tr>
<td>Microsomes plus glycerol 3-phosphate</td>
<td>188</td>
</tr>
<tr>
<td>B. None</td>
<td>10</td>
</tr>
<tr>
<td>Microsomes</td>
<td>40</td>
</tr>
<tr>
<td>Microsomes plus glycerol 3-phosphate</td>
<td>63</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Products of palmityl-CoA metabolism in presence and absence of glycerol 3-phosphate and microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>The reaction mixture was as described in Fig. 1, except that unlabeled citrate was used, and the reaction time was 20 min. All tubes contained 2 mg of high speed supernatant protein and 62.5 μM 3H-palmityl-CoA (1.28 × 10^6 cpm per pmole). Where indicated, tubes also contained microsomes (1 mg of protein), and 9.2 mM D-glycerol 3-phosphate. In the analytical procedure used (17), unchanged palmityl-CoA is not recovered.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Additions</th>
<th>Radioactivity recovered in</th>
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<tbody>
<tr>
<td></td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>None</td>
<td>37</td>
</tr>
<tr>
<td>Microsomes</td>
<td>14</td>
</tr>
<tr>
<td>Microsomes plus glycerol 3-phosphate</td>
<td>1</td>
</tr>
</tbody>
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DISCUSSION

Fatty acid synthesis in vivo is stimulated by the addition of glycerol 3-phosphate and microsomes to the high speed supernatant enzymes (Figs. 1 to 6). Addition of palmityl-CoA results in an inhibition of fatty acid synthesis which can be partially relieved by the addition of glycerol 3-phosphate and microsomes (Fig. 7). The evidence that these findings may be of significance in vivo is as follows. The concentrations of long chain acyl-CoA in liver of rats on diets high in carbohydrate is between 30 and 50 μmole per g of fresh weight. It becomes elevated to 110 to 135 μmole per g of fresh weight when the animals are deprived of food or fed diets high in fat (5, 25). It is difficult to judge the effective physiological concentration of palmityl-CoA from these figures. Because of its soaplike nature, appreciable amounts of palmityl-CoA may be bound to various lipophilic cell constituents in vivo. The concentration of glycerol 3-phosphate in liver of rats on diets high in carbohydrate is between 0.5 and 1.3 μmole per g of fresh weight. It can be depressed to less than 0.1 μmole per g of fresh weight by starvation or by administration of epinephrine (25-28). The Kₘ of 0.37 mM for l-glycerol 3-phosphate, which was observed for the stimulation of fatty acid synthesis in vivo (Fig. 1), is thus in the middle of the physiological range of glycerol 3-phosphate concentrations.

The stimulating effect of glycerol 3-phosphate and microsomes can be elicited either when they are present from the start, or when they are added subsequent to starting the reaction (Fig. 6). This can be interpreted to show that, in the absence of glycerol 3-phosphate and microsomes, the high speed supernatant makes an inhibitory product so rapidly that the inhibition appears to be present from the start. The inhibitory product cannot be long chain acyl-CoA, because up to Point A in Fig. 6, a maximum of only 1.25 μmole of long chain acyl-CoA (expressed as palmityl-CoA) was made. This amount would have inhibited fatty acid synthesis by less than 10%, whereas the inhibition observed at Point A was 70%. Another interpretation is that the enzymes of fatty acid synthesis in the high speed supernatant are isolated in a partially inhibited form. It can be calculated from concentrations of long chain acyl-CoA in vivo (5), and from the amount of high speed supernatant protein in 1 g of fresh liver (9), that the maximum amount of endogenous long chain acyl-CoA introduced into the reaction mixture with 2 mg of high speed supernatant protein is less than 1 μmole. It follows that if the enzyme system in the high speed supernatant is present in a form that is inhibited 70% (Fig. 6), this inhibition cannot be caused by endogenous long chain acyl-CoA itself. Nevertheless, the inhibition can be reversed by the addition of glycerol 3-phosphate and microsomes. Thus, long chain acyl-CoA cannot cause an inhibition of fatty acid synthesis (Fig. 7), but it is not the only, or the immediate, cause of the inhibition.

Under certain conditions, the carboxylation of acetyl-CoA is the rate-limiting step of fatty acid synthesis in vitro (20, 30). Bortz and Lyman (4) found that palmityl-CoA behaves like a competitive inhibitor of acetyl-CoA carboxylase, with a K_i of 7 μM. Other workers (2, 5) have shown that incorporation of malonyl-CoA into fatty acids is also inhibited by palmityl-CoA. Fatty acid synthesis is therefore subject to feedback inhibition by end product at multiple points.

The process of fatty acid synthesis occurs while the growing chain of carbon atoms is attached to the acyl carrier protein of the fatty acid synthetase complex (31-36). Although ACP has not so far been isolated from animal sources, it has been shown that derivatives of bacterial ACP are active with animal enzymes (32). We wish to propose that the rate of fatty acid synthesis is dependent, among other things, on the concentration of free ACP available to initiate the synthesis of new chains. According to this proposal, transfer of the palmitoyl group from palmitoyl-CoA to ACP (Reaction 1) leads to a reduction in the concentration of free ACP, and hence in the rate of fatty acid synthesis. Considered kinetically, Reaction 1 would compete for ACP with acetyl transacylase (Reaction 2) and malonyl transacylase (Reaction 3).

\[
Palmityl-CoA + ACP \rightleftharpoons \text{palmitoyl-ACP} + \text{CoA} \quad (1) \\
Acetyl-CoA + ACP \rightleftharpoons \text{acetyl-ACP} + \text{CoA} \quad (2) \\
Malonyl-CoA + ACP \rightleftharpoons \text{malonyl-ACP} + \text{CoA} \quad (3)
\]

Reaction 1 would be shifted to the left by transfer of the palmitoyl group from CoA to glycerol 3-phosphate. This mechanism explains the competitive inhibition by palmitoyl-CoA of acetyl-CoA utilization, and the need for preincubating the enzyme system with palmitoyl-CoA to produce the maximum inhibitory effect (4).

SUMMARY

1. Glycerol 3-phosphate stimulates fatty acid synthesis by cell-free preparations of liver and lactating mammary gland. The concentration of L-glycerol 3-phosphate which gives one-half of maximum stimulation is 0.37 mM. Comparison of this value with concentrations of glycerol 3-phosphate in vivo under various conditions indicates that glycerol 3-phosphate may exert a controlling influence on fatty acid synthesis in vivo.

2. The effect of glycerol 3-phosphate requires microsomes. Crossover experiments show that the capacity of microsomes to stimulate fatty acid synthesis in the presence of glycerol 3-phosphate is only slightly affected by the nutritional or hormonal state of the animal, whereas the rate of fatty acid synthesis by high speed supernatant varies greatly depending on the nutritional or hormonal state of the animal.

3. The inhibition of fatty acid synthesis by long chain fatty acyl coenzyme A is not the only, or the immediate, cause of inhibition. It is proposed that one of the rate-limiting factors of fatty acid synthesis may be the availability of free ACP carrier protein. This will depend, among other things, on the rate of removal of long chain fatty acyl groups from long chain fatty acyl carrier protein.

REFERENCES


1 The abbreviation used is: ACP, acyl carrier protein.
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