Regulation of Triacylglycerol Synthesis in the Liver

MODULATION OF DIACYLGLYCEROL ACYLTRANSFERASE ACTIVITY IN VITRO

(Received for publication, March 3, 1982)

Hendrik P. Haagsman, Catharina G. M. de Haas, Math J. H. Geelen, and Lambert M. G. van Golde

From the Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands

Acyl-CoA:1,2-diacylglycerol O-acyltransferase (EC 2.3.1.20) of rat liver microsomes could be inactivated in vitro by incubation with ATP, Mg\(^{2+}\), and a 105,000 × g rat liver supernatant. Of the nucleotides tested, ATP was the most effective in inactivating diacylglycerol acyltransferase.

The rate of this inactivation was not influenced by the addition of cAMP. Treatment of rat liver microsomes with high concentrations of the catalytic subunit of cAMP-dependent protein kinase did not result in the inactivation of diacylglycerol acyltransferase.

The activity of diacylglycerol acyltransferase was lower in microsomes isolated in the presence of fluoride (50 mm) than in control microsomes isolated from homogenates containing chloride (50 mm) instead of fluoride. Interestingly, the activity of diacylglycerol acyltransferase in microsomes isolated in the presence of fluoride and that in control microsomes were both reduced to the same level upon treatment of the microsomes with Mg\(^{2+}\), ATP, and 105,000 × g supernatant.

In order to investigate whether the inactivated diacylglycerol acyltransferase could be reactivated the microsomes were reisolated, washed, and subsequently incubated with 105,000 × g supernatant of rat liver. Microsomal diacylglycerol acyltransferase could indeed be reactivated by a factor present in the 105,000 × g supernatant. The activating factor appeared to be heat-labile, nondialyzable, and trypsin-sensitive. The reactivation of microsomal diacylglycerol acyltransferase was inhibited in the presence of fluoride (50 mm).

Both inactivation and reactivation of diacylglycerol acyltransferase appeared to be reversible processes; reactivated microsomal diacylglycerol acyltransferase could be inactivated again by incubation with Mg\(^{2+}\), ATP, and 105,000 × g supernatant and subsequently reactivated again in the presence of 105,000 × g supernatant.

These findings are consistent with a model in which microsomal diacylglycerol acyltransferase is interconvertible between catalytically inactive and active states, possibly via a phosphorylation-dephosphorylation mechanism.

The final step in the de novo synthesis of triacylglycerols in the liver takes place in the endoplasmic reticulum (1, 2) and involves the conversion of 1,2-diacyl-sn-glycerols into triacylglycerols. The enzyme that catalyzes this step, diacylglycerol acyltransferase, is the only enzyme that is exclusively involved in the formation of triacylglycerols. Diacylglycerols do not only serve as precursors for the synthesis of triacylglycerols but also for that of the nitrogenous phospholipids, phosphatidyethanolamines and phosphatidylcholines.

In view of the different functions of triacylglycerols and phospholipids, regulation of phospholipid and triacylglycerol synthesis at this branchpoint seems likely. Indeed, we recently found that exposure of hepatocytes to glucagon caused a decrease in the activity of microsomal diacylglycerol acyltransferase, whereas the activity of cholinephosphotransferase (EC 2.7.8.2), which also uses diacylglycerols as substrate, was not affected (3). In line with these observations, triacylglycerol synthesis was decreased upon incubation of hepatocytes with glucagon, while phospholipid synthesis was not influenced (4). Furthermore, incubation of hepatocytes in the presence of fatty acids leads to an enhanced triacylglycerol synthesis, whereas phospholipid synthesis is much less affected (5, 6).

This treatment stimulated the activity of diacylglycerol acyltransferase but did not affect that of cholinephosphotransferase (6).

Preliminary investigations revealed that diacylglycerol acyltransferase could be inactivated in a time-dependent way by Mg\(^{2+}\), ATP, and a 105,000 × g supernatant from rat liver (3).

In the present communication, data are presented which show that the activity of microsomal diacylglycerol acyltransferase can be modulated reversibly by reactions that are consistent with a phosphorylation-dephosphorylation mechanism.

**EXPERIMENTAL PROCEDURES**

**Materials**—[1-\(^{14}\)C]Palmitoyl-CoA (specific activity 2.18 TBq/mol) was obtained from the Radiochemical Centre, and CDP[methyl-\(^{14}\)C]choline (specific activity 1.48 TBq/mol) and 1-[\(^{14}\)C]glycerol 3-phosphate (specific activity 5.33 TBq/mol) were from New England Nuclear (Dreieichenhain, FRG). Tris, adenosine, CAMP, AMP, ADP, ATP, dATP, GTP, CTP, UTP, and GDP were supplied by Boehringer Mannheim (FRG). Catalytic subunit of cAMP-dependent protein kinase, protein kinase inhibitor (both from bovine heart), and \(\beta,\gamma\)-methylene ATP were purchased from Sigma. Silica G was obtained from Merck (Darmstadt, FRG), sucrose (analytical grade) was from BDH Chemicals Ltd. (Poole, England), and dichloroethyle was purchased from Serva (Heidelberg, FRG). \(\beta,\delta\)-Flavaspidic acid N-methyl-\(^{14}\)C]glycerol 3-phosphate was obtained from Huhtanenki Oy, Leiras (Turku, Finland). All other chemicals (analytical grade) were supplied by J. T. Baker Chemical Co. (Deventer, The Netherlands).

**Animals**—Male Wistar rats (175-200 g), which were fed ad libitum a stock pelleted diet, were used. The animals were routinely killed between 8 and 9 a.m. In some experiments 48-h starved rats were used which had free access to water.

**Preparation of Rat Liver Microsomes**—Rat livers were rinsed with

\(1 \text{ The abbreviation used is: Bq, becquerel (1 Bq = 1 disintegration/} s\).
ice-cold 0.9% NaCl solution, mixed, and homogenized (20%, w/v) with the aid of a Potter-Elvehjem homogenizer in 250 mm sucrose, 50 mm Tris-HCl, pH 7.4, and 5 mm diethiothreitol (medium I). In some experiments livers were divided into two parts. One part was homogenized in medium I containing in addition 50 mm NaCl and the other part in medium I with 50 mm NaF. The homogenates were centrifuged at 1,000 × g for 5 min. The resulting supernatants were centrifuged for 10 min at 20,000 × g. Microsomes were obtained by spinning the 20,000 × g supernatant for 60 min at 105,000 × g. In some experiments, microsomes were resuspended in medium I and pelleted again by centrifugation. Protein contents of the subcellular fractions were estimated by the method of Bradford (7). Freshly isolated microsomes were used in all experiments.

Inactivation of Microsomal Diacylglycerol Acyltransferase—Diacylglycerol acyltransferase was routinely inactivated by incubating rat liver microsomes (3–4 mg of protein/ml) with 10 mm MgCl₂, 5 mm ATP, and 105,000 × g supernatant from rat liver (8–12 mg of protein/ml) during 15 min at 25 °C in the presence of 50 mm NaF (added as last component). Inactivation of diacylglycerol acyltransferase does not occur in washed microsomes if MgCl₂ and ATP are added and 105,000 × g supernatant is omitted, which means that a cytosolic factor is needed for inactivation. After the inactivation procedure the microsomes were collected by centrifugation for 60 min at 105,000 × g and subsequently resuspended with the aid of a Potter-Elvehjem homogenizer in medium I.

Reactivation of Microsomal Diacylglycerol Acyltransferase—After inactivation as described in the preceding paragraph, the activity of microsomal diacylglycerol acyltransferase could be restored by incubation of the microsomes with 105,000 × g supernatant obtained from livers of fed rats at 25 °C. In some experiments, microsomes were chromatographed on Sephadex G-50 (medium) prior to the reactivation procedure. The 105,000 × g supernatant was desalted in some cases by Sephadex G-50 chromatography.

Measurements of Enzyme Activities—Diacylglycerol acyltransferase was assayed in the microsomal fraction, using endogenous diacylglycerols as substrates. Microsomes (0.3–0.4 mg of protein) were incubated at 37 °C in a medium which contained the following components: 50 mm potassium phosphate, pH 6.5, 1.0 mm diethiothreitol, 50 mm NaF, bovine serum albumin (5 mg/ml, dialyzed (8) and charcoal-treated (9)) and γ-[14C]palmitoyl-CoA (240 Bq/nmol) in a total volume of 0.5 ml. The incorporation of palmitoyl-CoA into triacylglycerols was a linear function of time up to 1.5 min. The incubations were terminated after 1 min by the addition of 2 ml of methanol:chloroform (2:1, v/v). After extraction of the lipids (10), the triacylglycerols were chromatographed on Silica G using petroleum ether (b.p. 40-60 °C):diethyl ether:acetic acid (80:20:2, v/v) as developing solvent. The triacylglycerol fractions, was scraped from the plate and developed in an atmosphere saturated with NH₃. The silica, containing the triacylglycerol fractions, was scraped from the plate and assayed for radioactivity. Cholinephosphotransferase was assayed in the microsomal fraction, using endogenous diacylglycerol acyltransferase, had no effect on the inactivation (Fig. 2). Earlier we reported that homogenization of livers in a medium containing 50 mm NaF yielded diacylglycerol acyltransferase activities that were lower than those of control microsomes isolated in a medium without fluoride present. A possible explanation for this observation could be that fluoride

### RESULTS

Inactivation of Diacylglycerol Acyltransferase—In agreement with an earlier report from our laboratory (3), diacylglycerol acyltransferase activity could be reduced rapidly by incubating microsomes in the presence of 5 mm MgCl₂, 1 mm ATP, and a 105,000 × g supernatant fraction from rat liver. During this inactivation, the levels of diacylglycerols in the microsomes remain unchanged. In preliminary experiments (3) we found some inactivation of diacylglycerol acyltransferase by incubation of microsomes with MgCl₂ and ATP in the absence of a 105,000 × g supernatant. However, if the microsomes were thoroughly washed (see under 'Experimental Procedures'), no inactivation of diacylglycerol acyltransferase was observed in the presence of MgCl₂ and ATP alone.

ATP can be replaced by ADP, GTP, and ITP, although inactivation was much less pronounced in the presence of these nucleotides (Table I). On the other hand, dATP, β,γ-methylene ATP, AMP, and adenosine were not able to replace ATP. Interestingly, the activity of diacylglycerol acyltransferase was enhanced 2-fold in the presence of CTP. It is important to emphasize that none of the nucleotides affected the assay of diacylglycerol acyltransferase activity (not shown).

Inactivation is dependent on the presence of 105,000 × g supernatant (Fig. 1). No inactivation of diacylglycerol acyltransferase occurred when microsomes were incubated with high concentrations of cAMP-dependent protein kinase (5–15 units/ml) in the presence of 10 mm MgCl₂ and 5 mm ATP. Inclusion of cAMP (10⁻³ or 10⁻⁴ M) in the inactivation medium had no effect on the rate of inactivation. Also, preincubation of the cytosolic fraction with cAMP for 10 min at 25 °C, before using this fraction to inactivate microsomal diacylglycerol acyltransferase, had no effect on the inactivation (Fig. 2).

### TABLE I

<table>
<thead>
<tr>
<th>Nucleotide specificity for inactivation of diacylglycerol acyltransferase</th>
<th>Activity (expressed as percentage of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>ATP</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>ADP</td>
<td>86 ± 1</td>
</tr>
<tr>
<td>AMP</td>
<td>136 ± 13</td>
</tr>
<tr>
<td>Adenosine</td>
<td>121 ± 10</td>
</tr>
<tr>
<td>dATP</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>β,γ-Methylene ATP</td>
<td>162 ± 19</td>
</tr>
<tr>
<td>GTP</td>
<td>70 ± 11</td>
</tr>
<tr>
<td>ITP</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>UTP</td>
<td>93 ± 5</td>
</tr>
<tr>
<td>CTP</td>
<td>208 ± 24</td>
</tr>
</tbody>
</table>

| Average of 4 incubations ± S.D. |

---

**Fig. 1. Effect of increasing amounts of 105,000 × g supernatant on the inactivation of diacylglycerol acyltransferase (DGAT).** Microsomes (0.85 mg of protein) were inactivated in the presence of 10 mm MgCl₂, 5 mm ATP, and variable amounts of 105,000 × g supernatant. Supernatant was chromatographed on Sephadex G-50 before use. Inactivations were carried out for 15 min at 25 °C in a total volume of 0.25 ml. Immediately thereafter, diacylglycerol acyltransferase activity was measured.
would prevent the action of phosphoprotein phosphatases which would bring the enzyme in a more active state. If indeed a phosphorylation-dephosphorylation mechanism exists that regulates diacylglycerol acyltransferase activity, the fluoride-homogenized livers would contain diacylglycerol acyltransferase that is more phosphorylated. In that case, inactivation of diacylglycerol acyltransferase in control microsomes most eventually lead to the same activities as those found after inactivation of microsomes isolated in the presence of fluoride.

FIG. 2. Lack of cAMP to affect inactivation of microsomal diacylglycerol acyltransferase (DGAT). 105,000 × g supernatant was incubated for 10 min at 25 °C in the presence of cAMP (10⁻⁴ M). Subsequently the supernatant was used to inactivate microsomal diacylglycerol acyltransferase. Microsomes (1.67 mg of protein) were incubated in the presence of 10 mM Mg²⁺ and 5 mM ATP with control (○) or cAMP-treated (●) 105,000 × g supernatant (10.2 mg of protein/ml). After the indicated periods of incubation time (25 °C) in a total volume of 0.5 ml, the diacylglycerol acyltransferase activity was determined immediately.

FIG. 3. Effects of fluoride in the homogenization medium on the inactivation of diacylglycerol acyltransferase (DGAT) in microsomes derived from ad libitum fed and 48-h starved rats. Livers from each group of rats (3 rats per group) were divided into two parts. One part was homogenized in medium I + 50 mM NaCl, the other part in medium I + 50 mM NaF. After isolation of the microsomes, diacylglycerol acyltransferase was inactivated (in the presence of fluoride (50 mM) in both preparations), immediately followed by the diacylglycerol acyltransferase assay. For all inactivations, 105,000 × g supernatants from livers of fed rats were used. Zero time values of diacylglycerol acyltransferase activities were 82, 86, and 66 pmol-min⁻¹ per mg of protein in A (fed rats, livers homogenized in the presence of Cl⁻), 94, 30, and 56 pmol-min⁻¹ per mg of protein in B (fed rats, livers homogenized in the presence of F⁻), 40, 40, and 90 pmol-min⁻¹ per mg of protein in C (starved rats, livers homogenized in the presence of Cl⁻), and 32, 25, and 43 pmol-min⁻¹ per mg of protein in D (starved rats, livers homogenized in the presence of F⁻). S.D. of the other time points were calculated via the S.D. of the percentage decrease compared to the zero time value of each rat.

FIG. 4. Reactivation of diacylglycerol acyltransferase (DGAT). A, diacylglycerol acyltransferase was firstly inactivated as described under "Experimental Procedures." Subsequently, after reisolation of the microsomes (by centrifugation for 1 h at 105,000 × g), diacylglycerol acyltransferase was activated by incubation with the cytosolic fraction (10.0 mg of protein/ml) in the presence (○) or absence (●) of 50 mM NaF at 25 °C, immediately followed by the diacylglycerol acyltransferase assay. ○, basal activation (i.e. activation in the presence of medium I). Microsomal protein concentration in the activation was 4.3 mg/ml. B, microsomes, incubated for 15 min with only 10 mM Mg²⁺ and 105,000 × g supernatant, were resuspended and treated like the inactivated microsomes as described above. Microsomal protein concentration was 5.7 mg/ml. Data are from one experiment out of 3 experiments with similar results.

FIG. 5. Activation of diacylglycerol acyltransferase (DGAT) as a function of the amount of added 105,000 × g supernatant. After inactivation of microsomal diacylglycerol acyltransferase (see under "Experimental Procedures") the microsomes were reisolated. Subsequently the microsomes (3.4 mg/ml) were incubated for 15 min at 25 °C with various amounts of 105,000 × g supernatant. Immediately hereafter, diacylglycerol acyltransferase activity was determined.
Modulation of Diacylglycerol Acalytransferase Activity

TABLE II
Effects of various treatments of the cytosolic fraction on the activation of diacylglycerol acyltransferase

Microsomal diacylglycerol acyltransferase was inactivated as described under "Experimental Procedures." After reisolation, microsomes (3.5 mg of protein/ml) were incubated for 15 min at 25 °C with various additions. Activity of diacylglycerol acyltransferase at t = 0 was 28 pmol-min⁻¹ per mg of microsomal protein.

<table>
<thead>
<tr>
<th>Additions</th>
<th>pmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>67</td>
</tr>
<tr>
<td>S₁₀⁵, 0.4 mg of protein/ml</td>
<td>244</td>
</tr>
<tr>
<td>S₁₀⁵, treated for 5 min at 100 °C (0.4 mg of protein/ml)</td>
<td>74</td>
</tr>
<tr>
<td>S₁₀⁵, treated for 60 min at 37 °C (0.4 mg of protein/ml)</td>
<td>365</td>
</tr>
<tr>
<td>S₁₀⁵, stored for 9 days at 4 °C (0.3 mg of protein/ml)</td>
<td>672</td>
</tr>
<tr>
<td>S₁₀⁵, trypsin-treated (0.4 mg of protein/ml)</td>
<td>66</td>
</tr>
</tbody>
</table>

*Values are from a representative experiment with duplicate assays. The different experiments were repeated 2–3 times with similar results.

S₁₀⁵ = 105,000 × g supernatant, gel filtered on Sephadex G-50.

Trypsin-treated; S₁₀⁵ was incubated for 60 min at 37 °C with trypsin (1:25, w/w), whereafter trypsin inhibitor was added before incubation with microsomes.

Indeed, incubation of control microsomes with Mg²⁺, ATP, and 105,000 × g supernatant for 10 min reduces the activity of diacylglycerol acyltransferase to the same value as that measured in microsomes isolated in the presence of fluoride (Fig. 3). This does not only hold for rats fed ad libitum but also for rats that were starved for 48 h. These findings suggest a connection between the Mg-ATP-dependent inactivation of diacylglycerol acyltransferase and the prevention of diacylglycerol acyltransferase activation during homogenization in the presence of fluoride.

Reactivation of Diacylglycerol Acalytransferase—Fig. 4A shows that inactivated diacylglycerol acyltransferase could be reactivated by incubating the microsomes in the presence of a 105,000 × g supernatant. Fluoride (50 mM) inhibited this reactivation. Interestingly, microsomes which had not been inactivated before could not be activated (Fig. 4B).

Reactivation was dependent on the amount of 105,000 × g supernatant protein added (Fig. 5).

Sephadex G-50 chromatography of the 105,000 × g supernatant did not affect the reactivating potential. In addition, during conventional bag dialysis the activating factor was retained in the bag. The activating factor disappeared when the supernatant was boiled for 5 min (Table II). Also trypsin treatment resulted in a disappearance of the activating factor.

On the other hand, incubation of the 105,000 × g supernatant...
which uses membrane-bound diacylglycerols as substrates and glycerol acyltransferase is an integral membrane protein phor-ylation-dephosphorylation mechanism. Because diacyl-
catalytically inactive and active states, possibly via a phos-
diacylglycerol acyltransferase activity is regulated between are min line with an already suggested mechanism (3), in which acyltransferase activity in these microsomes (Fig. 3).

Diacylglycerol acyltransferase was in-
promounced after preincubation of the microsomes with Mg 2+ , 
renounced again (Fig. 6).

The following experiment was designed to investigate this 
question. Microsomal diacylglycerol acyltransferase was in-
activated by incubation with Mg 2+ , ATP, and 105,000 × g supernatant and subsequently reactivated by treatment with 105,000 × g supernatant as described in Fig. 4. After the reactivation, the microsomes were resolated and incubated again with Mg 2+ , ATP, and supernatant. Fig. 6 shows that this treatment decreased the activity of diacylglycerol acyltransferase again to the same value as that observed after the first inactivation reaction. After resedimentation and reisolation in medium I, diacylglycerol acyltransferase could be reactivated again (Fig. 6).

The results shown in Fig. 4 suggested that activation of microsomal diacylglycerol acyltransferase is much more pronounced after preincubation of the microsomes with Mg 2+ , ATP, and 105,000 × g supernatant (to inactivate the enzyme) than for control microsomes. Diacylglycerol acyltransferase could be activated directly, i.e. without prior inactivation, in microsomes from livers homogenized in the presence of fluo-
ride and in microsomes from livers of fasted rats (data not shown). This is probably due to the lower diacylglycerol acyltransferase activity in these microsomes (Fig. 3).

**DISCUSSION**

The results presented here demonstrate that the activity of diacylglycerol acyltransferase in rat liver microsomes can be modulated in a reversible way. Until so far these observations are in line with an already suggested mechanism (3), in which diacylglycerol acyltransferase activity is regulated between catalytically inactive and active states, possibly via a phos-
phylation-dephosphorylation mechanism. Because diacyl-
glycerol acyltransferase is an integral membrane protein which uses membrane-bound diacylglycerols as substrates and has not been purified until now, it is very difficult to determine whether diacylglycerol acyltransferase is a protein that indeed can be functionally phosphorylated. Nevertheless, the results suggest that diacylglycerol acyltransferase can be inactivated in a mode consistent with a phosphorylation mechanism.

This inactivation requires ATP, although some inactivation occurs in the presence of ADP, GTP and ITP. AMP, adeno-
sine, dATP, and β, γ-methylene ATP were ineffective in re-
placing ATP in the inactivation reaction. The fact that β, γ-
methylene ATP cannot replace ATP suggests that cleavage of a phosphate group may play a role in the inactivation reaction. No satisfactory explanation can as yet be offered for the stimulation of diacylglycerol acyltransferase activity by CTP in the inactivation reaction. Like the other nucleotides tested, CTP did not affect the assay of diacylglycerol acyltransferase activity.

Previous studies (3) indicated that diacylglycerol acyltransferase activity can be decreased upon incubation of hepatocytes in the presence of glucagon or cAMP. Yet, in freshly isolated microsomes cAMP had no effect on the inactivation of the enzyme. It is of course unknown at which level cAMP affects diacylglycerol acyltransferase activity.

Some enzymes that are inactivated by a phosphorylation mechanism require cAMP-independent protein kinases in the inactivation reaction (13). An example in lipid metabolism is the microsomal enzyme hydroxymethylglutaryl-CoA reductase (EC I 1.1.3.4), which is thought to be an important regulat-
ory enzyme in cholesterol biosynthesis. This enzyme is regulated by reversible phosphorylation (14). Dibutylryl cAMP and glucagon diminish hydroxymethylglutaryl-CoA reductase activity in isolated hepatocytes (15, 16). Yet, the enzyme is not a substrate for cAMP-dependent protein kinase but is inactivated by a specific reductase kinase (17).

Another finding that points in the direction of a phosphor-
ylation-dephosphorylation mechanism is that the presence of fluoride, an anion known to inhibit phosphoprotein phosphatases, in the homogenization medium results in a lower diacylglycerol acyltransferase activity. A similar finding was ob-
erved with hydroxymethylglutaryl-CoA reductase (18, 19). Activation would not take place during homogenization under these conditions. Interestingly, diacylglycerol acyltransferase in microsomes isolated from livers of fed and 48-h starved rats in the presence of fluoride could be inactivated to the same end values as diacylglycerol acyltransferase from control mi-
cromes. This suggests that fluoride protects against dephos-
phorylation during homogenization.

Inactivated diacylglycerol acyltransferase could be acti-
ated upon incubation with a 105,000 × g supernatant from rat liver, although always some basal activation was observed during incubation at 25 °C in the absence of supernatant (see Figs. 4 and 6 and Table III). As pointed out earlier (3), activation was dependent on the presence of diamethylolethritol in the medium. Fluoride inhibited this activation, again suggest-
ing that a phosphatase is the activating factor. The activating factor was not dialyzable, heat-labile, and trypsin-sensitive, indicating that it is a protein. Interestingly, the factor can be activated upon storage at 4 °C. It is not likely that soluble phosphatidic acid phosphohydrolase is the activating factor in the cytosolic fraction, because this enzyme loses its activity upon storage at 4 °C (Table III). In addition, phosphatidic acid levels in microsomes are low, and consequently microsomal diacylglycerol levels will not be affected by incubation with the cytosolic fraction. Indeed no change in microsomal diacylglycerol levels could be observed. The conversion of diacylglycerols into triacylglycerols was reported to be stimu-
lated by fatty acid binding protein (20). However, the activ-
ation of diacylglycerol acyltransferase could not be inhibited

<table>
<thead>
<tr>
<th>Additions</th>
<th>Diacylglycerol acyltransferase activity</th>
<th>Phosphatic acid phosphohydrolase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (basal activation)</td>
<td>109</td>
<td>2.05</td>
</tr>
<tr>
<td>Sup (10.2 mg of protein/ml)</td>
<td>297</td>
<td>1.55</td>
</tr>
<tr>
<td>Sup (11.2 mg of protein/ml)</td>
<td>224</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Activation factor expressed as ability to reactivate diacylglycerol acyltransferase in activation reaction (15 min at 25 °C).

**TABLE III**

Effect of storage at 4 °C on the diacylglycerol acyltransferase activating factor and the activity of soluble phosphatic acid phosphohydrolase in 105,000 × g rat liver supernatant

105,000 × g supernatant from rat liver, either stored for 13 days at 4 °C or used freshly, were tested for their ability to reisolate diac-
ylglycerol acyltransferase and for the activity of soluble phosphatic acid phosphohydrolase. Starting value of diacylglycerol acyltrans-
ferase (determined after resolation of the microsomes): 44 pmol-min⁻¹
per mg of protein. Data are taken from a representative experiment carried out in duplicate.
Modulation of Diacylglycerol Acrlytransferase Activity
by flavaspidic acid, an inhibitor of the fatty acid binding protein (21).²

Like inactivation, activation of diacylglycerol acyltransferase is a reversible process, indicating that this enzyme can be regulated rapidly by interconversion between metabolically active and inactive states. Diacylglycerol acyltransferase activity has not reached its maximum after 15 min of incubation in the presence of 105,000 × g supernatant. It appears that diacylglycerol acyltransferase has a large reserve capacity which can be used when for example the fatty acid concentration is high which results in a higher diacylglycerol acyltransferase activity and a higher rate of triacylglycerol synthesis (6).

Work is in progress to purify the activator protein and inactivation factor. Furthermore, we will investigate the physiological significance of the regulation of diacylglycerol acyltransferase activity as described in this report.

REFERENCES
8. Hanson, R. W., and Ballard, F. J. (1968) J. Lipid Res. 9, 667-668

Regulation of triacylglycerol synthesis in the liver. Modulation of diacylglycerol acyltransferase activity in vitro.
H P Haagsman, C G de Haas, M J Geelen and L M van Golde


Access the most updated version of this article at http://www.jbc.org/content/257/18/10593.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/257/18/10593.citation.full.html#ref-list-1