Hepatic Monoacylglycerol Aciyltransferase Is Regulated by sn-1,2-Diacylglycerol and by Specific Lipids in Triton X-100/Phospholipid-mixed Micelles

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The lipid cofactor requirement of hepatic monoacylglycerol acyltransferase (MGAT) (EC 2.3.1.22) was studied in Triton X-100/lipid-mixed micelles. Anionic phospholipids and anionic lysophospholipids stimulated MGAT activity, whereas fatty acids and sphingosine inhibited enzyme activity. Phosphatidic acid was a potent activator, stimulating MGAT 11-fold at 4.2 mol %. Kinetic studies revealed that phosphatidic acid, with an apparent $K_m$ of 0.26 mol %, was a better activator than phosphatidylserine, phosphatidylinositol, or cardiolipin. Of the anionic lysophospholipids, lysophosphatidic acid was a better activator than lysophosphatidylerine, stimulating maximally at less than 3 mol %. Oleate was a more potent inhibitor ($K_i$, 2.4 mol %) than sphingosine ($K_i$, 18.3 mol %). The dependence of MGAT on sn-2-monacylglycerol was not cooperative in the absence or presence of anionic phospholipids, oleic acid, or sphingosine. The apparent $K_m$ for sn-2-monO18:1-glycerol was 1.24 mol % in the presence of maximally activating phospholipid and 0.19 mol % when phospholipid was omitted. MGAT's product sn-1,2-diacylglycerol was a weaker activator than the anionic phospholipids, but the effects of diacylglycerol and phospholipid were additive. Activation by sn-1,2-diC18:1-glycerol was highly cooperative with a Hill coefficient of 3.6. Activation was specific for the sn-1,2-stereoisomer; neither 1,3-diacylglycerol nor other analogs of sn-1,2- or 1,3-diacylglycerol were activators. Since several of the lipid modulators of MGAT activity are intracellular second messengers, these data suggest the possibility that regulatory links exist between signal transduction and the synthesis of complex lipids via the monoacylglycerol pathway.

Rat hepatic monoacylglycerol acyltransferase (EC 2.3.1.22) (MGAT) is a developmentally expressed microsomal enzyme whose specific activity in suckling rats is as much as 700-fold higher than the activity in adults (1). The enzyme catalyzes the stereospecific synthesis of sn-1,2-diacylglycerol from sn-2-monoacylglycerol and long chain fatty acyl coenzyme A (1, 2). MGAT may play an important role in regulating glycerolipid synthesis because its sn-2-monoacylglycerol substrate is a competitive inhibitor of glycerol-3-phosphate acyltransferase (3) and diacylglycerol kinase (4) and because its sn-1,2-diacylglycerol product is both an intermediate in phospholipid and triacylglycerol synthesis and an activator of protein kinase C. Hepatic MGAT has an increased affinity for sn-2-monoacylglycerols that contain polyunsaturated acyl chains (5, 6). Thus, the high MGAT activity observed in liver from neonatal rats, hibernating marmots, developing chick embryo, and streptozotocin-induced diabetic rats (5, 7) may function to retain essential fatty acids during physiologic periods characterized by massive lipolysis. MGAT also has an inferred role in diacylglycerol-mediated signal transduction pathways (8, 9) and in the incorporation of xenobiotics into glycerolipids (10, 11). In the intestine, MGAT provides a major route for the synthesis of chylomicra triacylglycerol (12–14). Despite the importance of MGAT in hepatic and intestinal lipid metabolism, little is known about the enzyme's structure and regulation.

Detailed characterization of enzymes like MGAT that synthesize glycerolipids has been limited. These enzymes are integral membrane proteins and difficult to study because their substrates and products are hydrophobic or amphipathic. Enzyme assays that use organic solvents to disperse hydrophobic constituents typically involve nonhomogeneous mixtures of enzyme, activators, substrates, and phospholipids which render kinetic analyses problematic. Many of these difficulties can be overcome by the use of detergent/phospholipid-mixed micellar assays. Such assays have been used successfully to examine the lipid and ion dependencies of several membrane-bound enzymes (15, 16). For review, see Ref. 17. Use of the mixed micellar assay allows one to manipulate the concentrations of enzyme, substrate, and lipid in a precisely defined environment that obeys surface dilution kinetics (18).

We employed mixed micellar assays in order to determine whether MGAT activity has an absolute requirement for lipids, to define the stoichiometry and specificity of the lipid requirement, and to elucidate the mechanism of the lipid dependence. We found that negatively charged phospholipids and sn-1,2-diacylglycerol activated MGAT and that fatty acids and sphingosine inhibited MGAT activity. These results suggest the possibility that regulatory links exist between signal transduction and the synthesis of complex lipids via the monoacylglycerol pathway.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were reagent grade. Phosphatic acid (from egg lecithin), dioleoylphosphatidylcholine, phosphatidylcholine (pig liver), phosphatidylethanolamine (pig liver), phosphatidylserine (boar brain), phosphatidylcholine (pig liver), lysophosphatidic acid (from egg lecithin), dioleoylphosphatidylglycerol, lysophosphatidylcholine (pig liver), lysophosphatidylserine (boar brain), 4OCl8:1 phosphatidyli-
glycerol, lysophosphatidylethanolamine (pig liver), sphingomyelin (beef brain), ceramide (beef brain), neutral lipids, cerebroside (beef brain), cardiolipin (beef heart), fatty acids, and sphingosine (beef brain) were purchased from Serdary Research Laboratories, Inc. DiC18:1-phosphatidylerine, diC4:0-phosphatic acid, diC18:2-phosphatic acid, and diC18:2-phosphatic acid were from Avanti Polar Lipids. BSA (serum-free) was from Triton X-100, DTI, EDTA, l-glycerol 3-phosphate, dicydroxycetone phosphate, and CM-Sepharose FF were from Sigma. QAE-Sephadex and hydroxylapatite (Bio-Gel HTP) were from Pharmacia LKB Biotechnology Inc. and Bio-Rad, respectively. Thin layer chromatography plates were from Analtech.

**Partial Purification of MGAT**—Animal use was approved by the Animal Care Committee of University of North Carolina. Pregnant Sprague-Dawley rats were obtained from Zivic-Miller and housed in the University of North Carolina animal facility under controlled 12-h light cycles with free access to food and water. Within 24 h after birth, each litter was culled to a maximum of 12 pups. On the 11th day after birth, studies were initiated to purify the enzyme to apparent homogeneity. However, because the final purification steps required transfer into CHAPS which inhibited more than 90% of the enzyme activity (data not shown), we studied MGAT's lipid dependence with preparations purified only through the hydroxylapatite step. To determine whether the hydroxylapatite-purified enzyme preparation contained phospholipids, material was extracted into CHCl3, and phospholipids were separated by thin layer chromatography on Silica Gel G plates in heptane/isopropyl ether/25% water (5:5:4, v/v/v). The material remaining at the origin was eluted three times with CHCl3/methanol (1:2, v/v). Phospholipid was quantified by measuring inorganic phosphorus following perchloric acid digestion. No phosphate was detected in either of two independent enzyme preparations.

The solubilized enzyme preparation was stored in aliquots at −70 °C in a buffer containing 20% glycerol, 5% ethylene glycol, 20 mM MES, pH 6.5, 1 mM EDTA, 1 mM DTI, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/mL pepstatin, 0.5 mM benzamidine, 0.2% Triton X-100, and 0.2 M NaCl. Hydroxylapatite-purified MGAT specific activities were 1443, 1422, 1423, and 2361 nmol/min/mg from four different purified preparations when MGAT was assayed in a 0.2-mL reaction mixture containing 100 mM Tris-Cl, pH 8, 1 mg/mL BSA, 450 μg/mL sonicated microsomal lipids, 150 μM sn-2-monoC18:1-glycerol and 300 μM sn-1,2-diC18:1-glycerol dispersed together in 10 μL of acetone, 5 mM DTI, 2.5 mM EDTA, 25 mM Hepes (pH 7.5), 1 mM EDTA, and 0.1–0.5 μg of protein (6).

**MGAT Mixed Micellar Assay**—Stock solutions (stored in chloroform or hexane) of the different lipids employed were dried under a stream of nitrogen in a glass tube and solubilized in Triton X-100 by vortex mixing and brief sonication at room temperature. Required amounts of lipids were then added to the reaction mixture. MGAT activity was assayed at 23 °C in a 0.2-mL reaction mixture that contained 100 mM Tris-Cl, pH 7.0, 0.5 mg/mL BSA, 150 μM sn-2-monoC18:1-glycerol, 0.22% Triton X-100 (3 mM micelle concentration), 25 μM [3H]Palmitoyl-CoA, 0.25–0.5 μg of hydroxylapatite-purified protein, and the indicated concentrations of specific phospholipids. After a 10-min incubation, the products were extracted into heptane, and an aliquot was counted (20). For some studies, the remaining portion of the heptane extract was concentrated in a Savant Speedvac concentrator and chromatographed with carrier lipids on a 10-cm Silica Gel G plate in heptane/isopropanol ether/acetic acid (60:40:4, v/v/v). Lipids were visualized by exposure to I2 vapor, and the areas corresponding to diacylglycerol and triacylglycerol were scraped and counted. Because more than 97% of the labeled product was diacylglycerol, thin layer chromatography was not routinely performed. All assays contained optimal amounts of sn-2-monoC18:1-glycerol and [3H]Palmitoyl-CoA and measured initial rates. For each study reported, data are shown for a representative experiment that was repeated two to six times using two to four different micellar preparations.

**Other Methods**—Protein was measured by the method of Lowry et al. (21) with BSA as the standard. When the samples contained Triton X-100, protein was precipitated with 10% trichloroacetic acid before protein was measured. sn-1,2,DiC18:1:Glycerol and sn-1,3,DiC18:1:

2 For purposes of calculating mole fractions, the aqueous micromolar concentration of Triton X-100 (0.3 mM), was subtracted from the total detergent concentration. Final concentrations include the Triton X-100 that is added together with the purified enzyme.

**RESULTS**

**MGAT Activity in Triton X-100-mixed Micelles**—Triton X-100-mixed micelles were used to provide an inert surface in which hydrophobic substrates and potential lipid activators and inhibitors were dispersed in physically defined amounts, thereby providing a matrix that mimics the microsomal membrane bilayer. Compared with other detergents tested, Triton X-100 inhibits MGAT activity least and is most effective in solubilizing MGAT activity (6). The average number of lipid molecules present in a mixed micelle was estimated from the detergent aggregation number of 140 for Triton X-100 and the mol % concentration of the lipid present (17).

To determine the optimal detergent concentration for the mixed micelle assays, we measured MGAT activity at Triton X-100 concentrations ranging from 0.2 to 8 mM (with the monomer concentration subtracted), both in the presence and absence of added phosphatidylcholine and phosphatidylethanolamine, each at 112.5 μg/mL. We chose this phospholipid combination as the standard activator because it had previously been routinely used to stimulate both intestinal and neonatal hepatic MGAT activities in microsomes (12, 19). In the absence of phospholipid, Triton X-100 inhibited MGAT activity at all concentrations tested; when phospholipid was present, enzyme activity was stabilized at a Triton X-100 concentration of less than 1.75 mM (Fig. 1). Thus, phospholipids stimulated MGAT activity and also reduced the inhibition of the enzyme by Triton X-100. A Triton X-100 concentration of 3 mM was chosen for all subsequent experiments. This concentration inhibits MGAT activity less than 50% and provides sufficient micelles to ensure the presence of less than a single MGAT protein/micelle. DTI and EDTA, which increase MGAT activity in a non-micellar assay (6), had no effect on MGAT activity in micelles, and were omitted. The divalent cations, Mg2+, Ca2+, Ba2+, Zn2+, and Mn2+, did not alter activity. BSA at 0.5 mg/mL increased basal enzyme activity 233% (data not shown) and was routinely included. In the absence of added lipids, BSA did not stimulate MGAT activity. These modifications resulted in an assay which was proportional to the amount of protein added to 0.75 μg and with time to 20 min when 0.3 μg of protein was present (data not shown).
Phospholipid Cofactor Dependence—Since several phospholipids had previously been shown to stimulate MGAT activity in microsomes, we tested the ability of a variety of phospholipids to stimulate MGAT. In order to ensure that the structure of the mixed micelles was similar to the structure of micelles composed of Triton X-100 alone (26, 27), the total lipid concentration used in Triton X-100-mixed micelles was constant (26, 27).

Phospholipid cofactor dependence—Since several phospholipids had previously been shown to stimulate MGAT activity in microsomes, we tested the ability of a variety of phospholipids to stimulate MGAT. In order to ensure that the structure of the mixed micelles was similar to the structure of micelles composed of Triton X-100 alone (26, 27), the total lipid concentration used in Triton X-100/phospholipid-mixed micelles did not exceed 18 mol %. At this concentration, micelle size is constant (26, 27).

Phospholipids that had been purified from natural sources were tested individually (Fig. 2A). The most potent activator was phosphatidic acid. As little as 0.4 mol % of phosphatidic acid activated MGAT 5-fold. At 4.2 mol %, phosphatidic acid activated MGAT 11-fold. Thus, initial activation was observed with a single molecule of phosphatidic acid/micelle whereas full activation required about six molecules/micelle. Other anionic phospholipids at low concentrations also stimulated MGAT activity. Cardiolipin and phosphatidylserine at 5.1 and 7.5 mol %, respectively, activated MGAT 7.7- and 10.8-fold. Phosphatidylinositol at 15.5 mol % stimulated enzyme activity to the same extent as phosphatidylserine. Synthetic diC18:1-phosphatidylglycerol stimulated MGAT activity 3.5-fold at 3.8 mol % with a pattern similar to that of phosphatidylserine (data not shown). Thus, the anionic phospholipids obtained from natural sources stimulated MGAT activity between 5- and 11-fold. Maximal stimulation with phosphatidic acid, cardiolipin, and phosphatidylserine occurred with 6, 7, and 10 molecules of the respective phospholipid/micelle. Only phosphatidylinositol required more than 22 molecules/micelle to stimulate MGAT maximally. In contrast, the zwitterionic phospholipids, phosphatidylincholine, phosphatidylethanolamine, and sphingomyelin were poor activators, requiring concentrations greater than 17 mol % to stimulate MGAT 4.5-5-fold.

To determine whether the acyl chains were needed for activity, we tested the ability of glycerol 3-phosphate and dihydroxyacetonephosphate and of specific lysophospholipids to activate MGAT. The water-soluble molecules did not alter MGAT activity (data not shown), suggesting that activators must be able to associate with micelles and, therefore, must be amphipathic or hydrophobic. The effect of added lysophosphatidic acid and lysophosphatidylserine was similar to the effect of phosphatidic acid and phosphatidylserine (Fig. 2B). Basal activity was stimulated 7- and 5-fold with 2.7 and 10.8 mol % of added lysophosphatidic acid and lysophosphatidylserine, respectively. In contrast, the zwitterionic lysophospholipids were less potent than their diacyl counterparts. Neither lysophosphatidylcholine nor lysophosphatidylethanolamine activated MGAT activity, even at concentrations as high as 11 mol %.

In order to test the specificity of the phospholipid acyl chain composition, synthetic phosphatidic acids of known composition were employed. When diC18:1-, diC18:2-, diC18:3-, and diC20:4-phosphatidylcholines were compared, the acyl chain length and degree of saturation had little effect on the ability of phosphatidylcholine to stimulate MGAT activity (Fig. 3A). The extent of stimulation by each of the synthetic phosphatidylcholines was similar to that observed with phosphatidylcholine purified from pig liver (Fig. 2A). Conversely, when we compared different synthetic phospholipids whose acyl chain composition was identical (Fig. 3B), phosphatidic acid and phosphatidylserine stimulated MGAT activity whereas phosphatidylethanolamine and phosphatidylcholine had little effect. These results were similar to the studies of the natural phospholipids (Fig. 2A). Thus, both the concentration dependencies and the relative ability of each species to activate MGAT indicate a high degree of specificity for anionic phospholipids (Fig. 2, A and B). Fig. 3C shows the effect of synthetic phosphatidic acids of known acyl composition. The effect of both diC18:1-phosphatidic acid and diC18:2-phosphatidic acid on MGAT activity was similar to that of natural phosphatidic acid from egg lecithin (Fig. 2A). The maximum stimulation by these two lipids was approximately 4.8-fold at 2.2 mol % diC14:0-phosphatidic acid activated MGAT maximally (4.4-fold) at 9.4 mol %. diC4:0-phosphatidic acid had the least stimulatory effect with only a 1.9-fold increase in enzyme activity at 4.6 mol %. Since the highly water-soluble diC4:0-phosphatidic acid stimulated MGAT activity very little, activation appears to require the presence of phosphatidic acid within the micelle. These data (Figs. 2 and 3) strongly suggest that MGAT activation depends critically on the phospholipid head group. Activation is affected little, if any, by the degree of saturation or the chain length of the acyl groups, although at least one acyl chain is required to anchor the anionic phospholipid head group in the micelle. These results also suggest that membrane fluidity would not alter MGAT activity substantially.

Effect of Diacylglycerol on MGAT Activity—In a non-micellar assay, hepatic microsomal lipids and sn-1,2-diacylglycerol stimulate MGAT approximately 2-fold (6). We wondered, therefore, whether sn-1,2-diacylglycerol might be a specific activator of the enzyme. When phospholipids were absent, added sn-1,2-diC18:1-glycerol at 18.7 mol % stimulated MGAT activity 5-fold (Fig. 4A). No activation occurred when less than 11 mol % of diC18:1-glycerol was present (15 molecules/micelle). Ninety-one % of the labeled product was diacylglycerol, and no activity was detected when 1,2-diacylglycerol was added in the absence
of sn-2-monoC18:1-glycerol, indicating that the added diacylglycerol was not being used as a substrate.

Activation of MGAT was specific for the sn-1,2-stereoisomer; neither sn-1,3-diC18:1-glycerol nor the ether analogs of sn-1,2- or 1,3-diC18:1-glycerol activated enzyme activity (Fig. 4A). Activation by sn-1,2-diC18:1-glycerol was highly cooperative with a calculated Hill constant of 3.6. In the absence of phospholipid, diacylglycerol activated maximally when more than 30 mol% of diC18:1-glycerol was present.

Effect of Neutral Lipids, Fatty Acids, and Sphingosine on MGAT Activity—In order to determine whether other non-phospholipid constituents of the microsomal membrane might be activators, we examined the effects on MGAT activity of a variety of neutral lipids and sphingolipids. The addition of trIC18:1-glycerol and cholesterol oleate (Fig. 5), and ceramides, cerebrosides, and cholesterol (data not shown) at concentrations as high as 40 mol % inhibited MGAT activity less than 30%. Oleic acid had a biphasic effect; when present at 5.3 mol %, it stimulated MGAT activity 1.6-fold, but at 18.4 mol %, it inhibited the activity 97% (Fig. 5). Inhibition by linoleic acid, linolenic acid, and arachidonic acid was similar to that of oleic acid (data not shown). Sphingosine was also a potent inhibitor of MGAT activity. At 17.5 mol %, sphingosine inhibited MGAT activity 93%. This inhibition was not merely due to counteracting the charge of the anionic phospholipids; sphingosine inhibited equally well in the presence of activating concentrations of diacylglycerol (data not shown) or when no activating phospholipid was present.

Effect of Phospholipid Activators on the Kinetics of MGAT Activity—In order to understand the mechanism of phospholipid activation, the monoaoylglycerol dependence of MGAT was examined at different concentrations of phosphatidic acid, cardiolipin, or phosphatidylserine (Fig. 6). Double-reciprocal plots of the sn-2-monoC18:1-glycerol dependence were linear at all concentrations of phosphatidic acid, cardiolipin, or phosphatidylserine studied, indicating that MGAT exhibited saturation kinetics with respect to the concentrations of the monoaoylglycerol substrate in the absence and presence of added.
phospholipids. The dependence of MGAT activity on sn-2-monoC18:1-glycerol was not cooperative; the calculated Hill coefficient of 0.9 did not vary significantly with the addition of different concentrations of phosphatidic acid, cardiolipin, or phosphatidylserine.

Addition of each of the phospholipids to the micelle assay increased both the apparent \( V_{\text{max}} \) and the apparent \( K_m \). One interpretation of these data is that the anionic phospholipids act at two different sites to alter activity, an allosteric site that increases the number of effective enzyme molecules, and a catalytic site that reduces the enzyme's affinity for its monoacyl-glycerol substrate. The ability of each lipid to inhibit MGAT was measured (Fig. 7). The ability of each lipid to inhibit MGAT was measured (Fig. 7).

**Effect of Lipid Inhibitors on the Kinetics of MGAT Activity**—In order to determine the mechanism of inhibition by oleic acid and sphingosine, the kinetics of inhibition were examined (Fig. 7). The ability of each lipid to inhibit MGAT was similar in the presence or absence of phospholipid (see Fig. 5); however, because MGAT activity was very low when phospholipids were absent, kinetic studies were performed in the presence of activating phospholipids. When phosphatidylcholine and phosphatidylethanolamine were present, oleic acid at low concentrations did not activate MGAT activity (data not shown). MGAT activity exhibited saturation kinetics with respect to sn-2-monoC18:1-glycerol in the absence or presence of oleic acid (Fig. 7A) or sphingosine (Fig. 7B) and was inhibited by oleic acid and sphingosine in a similar concentration-dependent manner at each sn-2-monoC18:1-glycerol concentration. The results obtained from double-reciprocal plots suggested that the inhibition by oleic acid or sphingosine is noncompetitive or mixed. Thus, each of these inhibitors may interact with sites that participate in both substrate binding and catalysis. A replots of slopes versus the concentration of each inhibitor was used to calculate apparent \( K_i \) values (Fig. 7, A and B, insets). The apparent \( K_i \) for oleic acid was 2.4 mol % and for sphingosine was 18.3 mol %. Based on these values, oleic acid was a more potent inhibitor of MGAT activity than was sphingosine. The calculated Hill coefficient at each oleic acid or sphingosine concentration did not vary significantly from the value of 1 (0.9 and 1.1).

Table I summarizes the kinetic constants for MGAT. Based on the apparent \( K_i \) values, phosphatidic acid was a more potent activator than was cardiolipin or phosphatidylserine. The apparent \( K_i \) values for these activators were higher than the apparent \( K_m \) value for the sn-2-monoC18:1-glycerol substrate because, in the absence of added lipids, MGAT activity was saturated at very low concentrations of sn-2-monoC18:1-glycerol. These studies suggest that added phosphatidic acid, phosphatidylserine, or cardiolipin each activate MGAT by a similar mechanism.
MGAT activity peaks on the sixth to ninth post-natal days and indicated concentrations. The data are plotted as greater than 0.971.

For sn-2-monoC18:1-glycerol in the absence of phospholipid.

Values are derived from data not shown elsewhere.

The activity of most membrane-bound enzymes is dependent on, or modulated by, the membrane lipids (31). Our understanding of how membrane-bound enzymes are regulated has been limited by difficulties in measuring the effects of phospholipid cofactors and lipophilic substrates in states that are physically undefined and non-homogeneous. The use of micellar assays can overcome these problems. Detergent-mixed micelles can effectively solubilize membrane-bound enzymes, thereby constituting a surface of known composition in which the enzyme can function (17). Use of mixed micelles facilitates the presentation of hydrophobic and amphipathic substrates, cofactors, or modulators to an enzyme in a physically defined system in which the kinetic properties can be studied. Including a low concentration of phospholipid in the detergent micelles does not greatly disrupt micellar structure (17, 26, 27), and endogenous lipids that are present in solubilized protein fractions are subject to surface dilution and can be effectively eliminated from consideration (17).

Since few microsomal activities of glycerolipid synthesis have been purified to homogeneity, information is still limited concerning their structure, their interaction with hydrophobic substrates within the plane of the membrane, and their regulation. Enzymes of glycerolipid metabolism that have been studied in mixed micelles include Escherichia coli diacylglycerol kinase (32), yeast diacylglycerol choline- and ethanolamine-phosphotransferase (33), yeast phosphatidylserine synthase and phosphatidylinositol synthase (15), cobra venom phospholipase A, (34), yeast phosphatidate phosphatase (16), rat liver CTP:phosphocholine cytidylyltransferase (35), and rat hepatic lipase (36). The specific lipids that modulate protein kinase C activity have also been analyzed (37, 38).

Purification of MGAT and the use of Triton X-100-mixed micelles now allows detailed characterization of enzyme activation and inhibition by lipid cofactors. MGAT exhibited Michaelis-Menten kinetics with respect to monoacylglycerol when the substrate concentration was expressed as a mole fraction of the mixed micelle. In the absence of phospholipid, the apparent K for sn-2-C18:1-glycerol was 0.19 mol %. When phosphatidylcholine and phosphatidylserine were both present, the apparent K was 1.24 mol %.

Phospholipid Cofactor Requirement—The data reported here clearly demonstrate that specific lipids modulated hepatic MGAT activity in mixed micelles. Although the lipid cofactor was not an absolute requirement for activity, MGAT could be activated as much as 11-fold by anionic phospholipids or by sn-1,2-diacylglycerol. The extent of activation by each phospholipid and lysophospholipid species differed. Anionic phospholipids were potent activators whereas zwitterionic phospholipids activated poorly. Kinetic studies demonstrated that phosphatidic acid was the best activator. Although the composition of the acyl chain did not appear to be critical, the presence of at
least one acyl chain was required, suggesting that activation would occur only if the anionic headgroup were anchored in the micelle. MGAT was activated in micelles that contained only a single molecule of phosphatidic acid, strongly suggesting that activation requires specific interactions of enzyme and anionic headgroup rather than merely the presence of a non-specific lipid annulus. The remarkable degree of specificity exhibited by different anionic phospholipids may be related to differences in physical properties of the anionic headgroups and suggests that phosphatidic acid and phosphatidylycerine could play specific regulatory roles in the synthesis of glycerolipids via the monoacylglycerol pathway.

The few other glycerolipid synthetic enzymes that have been studied in mixed micelles exhibit varying patterns of activation. Like MGAT, yeast phosphatidylycerine synthase is potently activated by phosphatidic acid in Triton X-100-mixed micelles (15). Although phosphatidylinositol also activated phosphatidylycerine synthase, both cardiolipin and sn-1,2-diacylglycerol were both competitive inhibitors (15). The yeast CPT1 and EPT1 gene products, diacylglycerol choline and ethanolamine phosphotransferase enzymes activated best by phosphatidylcholine, and phosphatidylinositol was a strong activator only of the cholinephosphotransferase (33). These differences between MGAT and other enzymes studied in mixed micelles suggest that their regulatory sites differ substantially.

**Diacylglycerol Effect**—The ability of MGAT’s reaction product, sn-1,2-diacylglycerol, to activate MGAT is of particular interest. The data suggest that an increase in the sn-1,2-diacylglycerol content of the endoplasmic reticulum would increase MGAT activity. Although the hepatic content of sn-1,2-diacylglycerol declines 65% in rats between postnatal days 1 and 10, the subcellular location of diacylglycerol in hepatocytes is not known (39). It is possible that diacylglycerol content varies in different subcellular membranes and that the endoplasmic reticulum content of diacylglycerol could increase independently.

Although sn-1,2-diacylglycerol was a less potent activator than the anionic phospholipids, the activation by sn-1,2-diacylglycerol was highly cooperative. The Hill coefficient of 3.6 suggests that sn-1,2-diacylglycerol can substitute for phospholipid activation when relatively high concentrations of diacylglycerol are present. In cellular membranes, however, it is likely that diacylglycerol would act to modulate the effect of anionic phospholipids.

Activation was stereospecific for sn-1,2-stereoisomer; the diacylglycerol analogs sn-1,3-diC18:1-glycerol, sn-2,3-diC18:1-glycerol ether and 1,3-diC18:1-glycerol ether did not affect MGAT activity. These specificity data suggest that sn-1,2-diacylglycerol interacts with a specific enzyme site rather than in a non-specific manner. Diacylglycerol inhibits phosphatidylycerine synthase from yeast, but it is not known whether inhibition is stereospecific (15). It was suggested that the elevated diacylglycerol content of stationary yeast cultures would block phospholipid synthesis, thereby favoring the synthesis of triacylglycerol (15). Although our data suggest that sn-1,2-diacylglycerol may increase glycerolipid biosynthesis via the monoacylglycerol pathway, definitive conclusions cannot be drawn without better information on the overall regulation of glycerolipid synthesis in neonatal liver.

Few reports exist on the regulation of MGAT by lipid modulators. Both phospholipids (1, 6, 20) and sn-1,2-diacylglycerol (6) stimulate neonatal hepatic MGAT activity in microsomal and solubilized preparations. In adult rat liver, lysophosphatidic acid and lysophosphatidylethanolamine stimulate MGAT activity measured using the activated xenobiotic substrates 3-phenoxybenzoyl-CoA or 2-hexadecylglycerol (10). In the case of 2-hexadecylglycerol, it was suggested that activation may have resulted from an improved dispersal of the emulsified substrate (10).

Our results strongly suggest that specific phospholipids, sn-1,2-diacylglycerol, fatty acids, and sphingosine could play a significant role in the cellular regulation of MGAT, thereby altering the overall flux of glycerolipid intermediates and the synthesis of triacylglycerol, phosphatidyglycine, and phosphatidylethanolamine. Phosphatidic acid is believed to be an intracellular second messenger (38, 40, 41), and several of the putative MGAT regulators, including phosphatidylycerine, fatty acids, and sphingosine also affect the activation of protein kinase C. Thus, the data presented here suggest the possibility that regulatory links might exist between signal transduction and the synthesis of complex lipids via the monoacylglycerol pathway.

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