ATP-independent Fatty Acyl-Coenzyme A Synthesis from Phospholipid

COENZYME A-DEPENDENT TRANSACYLATION ACTIVITY TOWARD LY SOPHOSPHATIDIC ACID CATALYZED BY ACYL-C OENZYME A:LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE*

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CoA-dependent transacylation activity in microsomes is known to catalyze the transfer of fatty acids between phospholipids and lysophospholipids in the presence of CoA without the generation of free fatty acids. We previously found a novel acyl-CoA synthetic pathway, ATP-independent acyl-CoA synthesis from phospholipids. We proposed that: 1) the ATP-independent acyl-CoA synthesis is due to the reverse reaction of acyl-CoAlyso phospholipid acyltransferases and 2) the reverse and forward reactions of acyltransferases can combine to form a CoA-dependent transacylation system. To test these proposals, we examined whether or not recombinant mouse acyl-CoA:1-acyl-sn-glycerol-3-phosphate (lysophosphatidic acid, LPA) acyltransferase (LPAAT) could catalyze ATP-independent acyl-CoA synthetic activity and CoA-dependent transacylation activity. ATP-independent acyl-CoA synthesis was indeed found in the membrane fraction from Escherichia coli cells expressing mouse LPAAT, whereas negligible activity was observed in mock-transfected cells. Phosphatidic acid (PA), but not free fatty acids, served as an acyl donor for the reaction, and LPA was formed from PA in a CoA-dependent manner during acyl-CoA synthesis. These results indicate that the ATP-independent acyl-CoA synthesis was due to the reverse reaction of LPAAT. In addition, bacterial membranes containing LPAAT catalyzed CoA-dependent acylation of LPA; PA but not free fatty acid served as an acyl donor. These results indicate that the CoA-dependent transacylation of LPA consists of 1) acyl-CoA synthesis from PA through the reverse action of LPAAT and 2) the transfer of the fatty acid moiety of the newly formed acyl-CoA to LPA through the forward reaction of LPAAT.

The fatty acid moieties of phospholipids are not static but are rather dynamically turned over under various conditions. Acyltransferases catalyze the acylation of lysophospholipids and are involved in the biosynthesis and fatty acid remodeling of phospholipids (1–6). In mammalian cells and tissues, five types of acylation reactions for lysophospholipids have been proposed, based on the acyl donor and requirement for cofactors, such as CoA. These comprise: 1) acyl-CoA:lysophospholipid acyltransferase, 2) the CoA-dependent transacylation system, 3) the CoA-independent transacylation system and 4) lysophospholipase/transacylase.

Acyl-CoA:lysophospholipid acyltransferases utilize acyl-CoA as an acyl donor and various lysophospholipids as acyl acceptors (1–8). Lands et al. (1, 7, 8) proposed that the acyl-CoA:lysophospholipid acyltransferases are involved in the accumulation of polyunsaturated fatty acids in phospholipids because they preferentially utilize polynsaturated fatty acid acyl-CoA as an acyl donor when 1-acyl-glycerophosphocholine (1-acyl-GPC); lysophosphatidylcholine, LPC) or 1-acyl-glycerophosphoinositol (1-acyl-GPI; lysophosphatidylinositol, LPI) serves as the acyl acceptor. It has been assumed that there are distinct acyl-CoA acyltransferases with different acyl acceptor specificities, although no conclusive data have been obtained yet.

A CoA-dependent transacylation system was first reported by Irvine and Dawson (9) in rat liver. In this system, fatty acids esterified in phospholipids are transferred to lysophospholipids in the presence of CoA without the generation of free fatty acids (9–15). In rabbit liver microsomes, arachidonic acid (20:4 n-6), linoleic acid (18:2 n-6), and stearic acid (18:0), esterified in phospholipids, are the main species transferred to lysophospholipids in the presence of CoA, indicating that this system shows strict fatty acid specificity (15). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) have been shown to serve as donor phospholipids, PI being the preferred acyl donor (14, 15).

The CoA-independent transacylation system catalyzes the transfer of fatty acids esterified at the sn-2 position of diacyl phospholipids to various lysophospholipids in the absence of any cofactors (13, 14, 16, 17). C20 and C22 polyunsaturated fatty acids being selectively transferred. The CoA-independent transacylation system is involved in the acylation of 1-O-alkyl-GPC (lysoplatelet-activating factor). Lysophospholipase catalyzes the hydrolysis of the carboxyl ester bonds of lysophospholipids (5, 6). Some lysophospholipase isoforms are known to catalyze a transacylation reaction between two LPC molecules, leading to the formation of a diacyl phospholipid PC and GPC.

* The abbreviations used are: GPC, sn-glycero-3-phosphocholine; GP, sn-glycero-3-phosphate; GPE, sn-glycero-3-phosphoethanolamine; GPI, sn-glycero-3-phosphoinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol-polyunsaturated fatty acid; IPTG, isopropyl-β-D-thiogalactopyranoside.

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Lysophospholipase/transacylase also does not require any cofactors such as CoA.

The properties of the CoA-dependent transacylation system, including the reaction kinetics and donor and acceptor selectivities, have been studied (3–6, 9–15). However, the mechanism underlying the CoA-dependent transacylation system is not fully understood yet. In fact, the identity of the enzymes responsible for CoA-dependent transacylation reactions has not been established. Although we and other investigators previously considered that acyl-CoA:lysophospholipid acyltransferases may be involved in CoA-dependent transacylation reactions (9, 10, 15, 18–20), direct and conclusive evidence has not been obtained because of the lack of success in purification and cloning of membrane-bound lysophospholipid acyltransferases and transacylases, including the CoA-dependent transacylation system.

Previously, we demonstrated ATP-independent and CoA-dependent acyl-CoA synthesis in microsomal fractions of mammalian tissues (19, 20). No appreciable acyl-CoA was formed from free fatty acids under the same experimental conditions, indicating that this acyl-CoA synthesis is distinct from the well known acyl-CoA synthetase activity that ligates fatty acids and CoA in the presence of ATP and Mg++. Lysophospholipids, such as LPC and LPI, are formed during acyl-CoA formation, suggesting a possible role of membrane phospholipids as fatty acyl donors. These findings prompt us to suggest that the reverse reactions of acyl-CoA:lysophospholipid acyltransferases are involved in CoA-dependent/ATP-independent acyl-CoA synthesis and that the CoA-dependent transacylation system consists of the combination of the reverse and forward reactions of the acyltransferases (Scheme 1).

Genetic and molecular biological approaches have led to a breakthrough in the field of acyltransferases. An Escherichia coli mutant lacking acyl-CoA:1-acyl-sn-glycero-3-phosphate (1-acyl-GP, lysophatidic acid) acyltransferase (LPAAT) was isolated (21), and the corresponding gene (plsC gene) was subsequently cloned (22). Very recently, mouse and human homologs of the type of human LPAAT. When the mLPAAT gene was expressed in JC201 E. coli cells lacking endogenous LPAAT activity (plsC mutant) (21), ATP-independent acyl-CoA synthetic activity was observed in the membrane fraction, with phosphatidic acid (PA) as the acyl donor. CoA-dependent synthesis of LPA from PA was also seen. These results suggest that mLPAAT can operate in reverse to form acyl-CoA and LPA. Furthermore, we observed CoA-dependent transacylation activity toward LPA in membranes containing mLPAAT. We conclude that the CoA-dependent transacylation activity toward LPA is due to the combination of the reverse and forward reactions of mLPAAT and suggest that other acyltransferase systems may involve a similar mechanism.

**EXPERIMENTAL PROCEDURES**

Materials—[1-14C]Linoleic acid (18:2, 2.0 GBq/mmol), 1-[1-14C]palmitoyl (16:0)-GPE (2.1 GBq/mmol), [U-14C]glycerol-labeled dioleoyl phosphatidic acid (5.33 GBq/mmol), and [3H]HIP (3H)myo-inositol 185 GBq/mmol) were purchased from PerkinElmer Life Sciences. LPA (1-oleoyl (18:1)-GP), LPC (1-palmitoyl (16:0)-glycerol-3-phosphocholine, 1-acyl-GP), LPE (1-acyl-glycerol-3-phosphoethanolamine, 1-acyl-GPE), LPI (1-acyl-glycerol-3-phosphoinositol, 1-acyl-GPI), PE and PI from bovine liver, essentially fatty acid-free bovine serum albumin, and fatty acids (18:0, 18:1, 18:2, and 20:4) were obtained from Sigma. CoA was obtained from Kyowa Hakko, Ltd. (Tokyo, Japan). TLC plates precoated with silica gel 60 (Type 5721) and silica gel with fluorescent indicator F254a (Type 5715) were from Merck. 14C-Labeled and unlabeled acyl-CoAs were synthesized by the method of Seedorf (23) with minor modifications. [1-14C]LPA (1-14C)6:0-GP and [3H]C5P (1-palmitoyl-2-3H)linoleoyl were prepared from [1-14C]6:0-GP and [3H]7C (1-palmitoyl-2-[3H]linoleoyl), respectively, by treatment with phospholipase D (Streptomyces chromofuscus; Roche Molecular Biochemicals). 1-[14C]6:0–2-14:0-PA, 1-[14C]6:0–2-16:0-PA, 1-[14C]6:0–2-18:0-PA, 1-[14C]6:0–2-18:1(n-9)-PA, 1-[14C]6:0–2-18:1(n-9)-PA, and 1-[14C]6:0–2-20:0-PA were enzymatically synthesized by acylation of 1-[14C]6:0-GP with the corresponding acyl-CoAs using mLPAAT. [3H]HIP ([3H]myo-inositol-labeled 1-acyl-GPI) was prepared from [3H]inositol-labeled PI by phosphatase P2 (Naja naja atra) treatment. All other reagents were of reagent grade.

Expression of mLPAAT in E. coli—The cDNA clone of mLPAAT was isolated as described previously (27). The whole open reading frame of clone 3 was excised and cloned into the pTrcHisC expression vector (Boehringer Mannheim, Germany) containing an N-terminal His6 fusion protein. The expression vector containing the mLPAAT sequence (pTrc/mLPAAT) was transfected into JC201 cells (pls C mutant). JC201 cells harboring pTrc/mLPAAT were cultured in LB medium at 30 °C until the A600 reached 0.6 and then cultured for an additional 3 h with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The pTrcHisC vector without an insert was used as a negative control. After induction with IPTG, bacterial cells were collected by centrifugation at 3,000 × g for 5 min and then disrupted by sonication in 0.25 mM sucrose, 1 mM EDTA, 0.1 mM Tris-HCl buffer (pH 7.4) containing a protease inhibitor mixture (Calbiochem). The lysate was centrifuged at 3,000 × g for 15 min to remove cell debris, and then the supernatant was further centrifuged at 105,000 × g for 60 min. The resulting membrane pellet was washed with the same buffer, and the final pellet was suspended in the same buffer and used for enzyme assays. The protein content was determined by the method of Lowry et al. (29) with bovine serum albumin as a standard.

Western Blotting—The expression of mLPAAT in E. coli was confirmed by Western blotting using a monoclonal antibody against the penta-His sequence epitope (Qiagen). Briefly, membrane fractions from JC201 cells harboring pTrc/mLPAAT or the absence of the insert or the presence of the absence of IPTG were separated by SDS-polyacrylamide gel electrophoresis (10% T, 2.6% C) and further blotted onto a nitrocellulose membrane (Protran BA85, Schleicher & Schuell). After blocking the membrane with 5% bovine serum albumin, it was incubated with monoclonal antibody against the HIS6 sequence epitope. Immunoreactive proteins were visualized by chemiluminescence using horseradish peroxidase-conjugated anti-mouse IgG (Medical & Biological Laborato-
Reverse Reaction of Acyltransferase

Effect of Mammalian LPAAT—\(\text{CoA}\)-dependent LPA Formation from PA—

Measurement of the Activity of LPAAT—Membrane fractions from JC201 cells expressing mLPAAT were incubated with 30 \(\mu\text{M}\) [\(^{14}\text{C}\)]linoleoyl-CoA (40,000 dpm) and 40 \(\mu\text{M}\) LPA (1-oleoyl (18:1 n-9)) in 50 mM Tris-HCl buffer (pH 7.4) for 30 min at 30 °C. After stopping the reaction, LPA was extracted by the method of Bligh and Dyer under acidic conditions (0.6 N HCl). LPA was separated by TLC developed with chloroform:methanol:acetic acid:water (65:25:4:2 v/v/v/v). The radioactivity was determined with an imaging analyzer or a liquid scintillation spectrometer.

Expression of mLPAAT in E. coli—First, we expressed mouse LPAAT in JC201 E. coli cells under regulation by the trc (trp-lac) promoter. E. coli plsc mutant JC201 lacks LPAAT activity (21). The expression of mLPAAT was examined by Western blotting using a monoclonal antibody against the penta-His sequence epitope. The molecular mass of mLPAAT was calculated to be 32 kDa from the cDNA sequence (27). As shown in Fig. 1A, there were no signals around 32 kDa for the membrane fractions from JC201 cells (lane 1) and JC201 cells harboring pTrc/mLPAAT but cultured in the absence of IPTG (lane 2). When JC201 cells harboring pTrc/mLPAAT were cultivated in the presence of 1 mM IPTG for 3 h, a 32-kDa protein was induced (lane 3), suggesting that mLPAAT was expressed. The expression of mLPAAT was further confirmed by the induction of LPAAT activity in the membrane fractions. There was no LPAAT activity in the membrane fraction from mock-transfected JC201 cells. In contrast, acylation of LPA to \([^{14}\text{C}]\)IPA in the presence of \([^{14}\text{C}]\)linoleoyl-CoA occurred when the mLPAAT gene was induced by IPTG (Fig. 1B). The results confirmed that mLPAAT encodes the protein responsible for \(\text{CoA}:\text{LPA} \text{acyltransferase activity.}

We examined the substrate specificity of mLPAAT expressed in JC201 cells using various lysophospholipids (Fig. 1C). ACP-dependent LPA synthesis toward 1-alkyl-ether-linked LPA (1-O-alkyl-GP) was approximately half of that toward 1-acyl type LPA (1-acyl-GP). However, other lysophospholipids, such as LPC (1-acyl-GPC, lyssolecithin), LPE (1-acyl-GPE), LPI (1-acyl-GPI), and lysoplatelet-activating factor (1-O-acyl-GPC), did not serve as acyl acceptor substrates.

We used various kinds of free fatty acids or acyl-CoA esters to define the acyl donor specificity of mLPAAT in terms of the chain length and degree of unsaturation. As shown in Fig. 1D, free fatty acid did not serve as an acyl donor. mLPAAT showed the highest activity with linoleoyl-CoA (18:2 n-6), palmitoyl-CoA (16:0), and myristoyl-CoA (14:0) and somewhat lower activity with arachidonoyl-CoA (20:4 n-6), oleoyl-CoA (18:1 n-9), and stearoyl-CoA (18:0). Thus, mLPAAT shows a broad specificity for acyl-CoAs.
We examined the acyl donor specificity of ATP-independent acyl-CoA formation. Various phospholipids, such as PA, PC, PE, or PI, and a free fatty acid (linoleic acid) were tested as acyl donors. The membrane fraction containing mLPAAT was incubated with 1 mM CoA and 1% bovine serum albumin in the presence of various lipids, and acyl-CoA formation was assessed as described under “Experimental Procedures.” As shown in Fig. 2D, acyl-CoA was formed only in the presence of PA. Other phospholipids (PC, PE, and PI) and the free fatty acid failed to serve as acyl donors. In the membrane fraction from control cells, acyl-CoA was not formed even in the presence of PA (Fig. 2D, lane 7). Various PA species with different fatty acid composition were able to serve as acyl donor in ATP-independent acyl-CoA formation (data not shown). These results indicate that it is the head group of PA, not the acyl chain structure, that determines the acyl donor activity.

**CoA-dependent Formation of LPA from PA by mLPAAT—** If acyl-CoA is formed through the reverse reaction of LPAAT, LPA should be concomitantly formed from PA in a CoA-dependent manner. We confirmed this. When the membranes from mock- or mLPAAT-transfected JC201 cells were incubated with [U-14C]glycerol-labeled dipalmitoyl-PA, radiolabeled LPA was formed in the absence of CoA, suggesting that some endogenous phospholipase A-like activity was present (−5 pmol/mg protein; Fig. 3A). In the membranes containing mLPAAT, the formation of LPA increased on the addition of 50 μM CoA (Fig. 3A). The stimulatory effect of CoA was not observed in the membranes from mock-transfected cells. Furthermore, the addition of both unlabeled LPA and CoA markedly increased the [14C]LPA formation, probably because the dilution of [14C]LPA by unlabeled LPA reduced the conversion of [14C]LPA to [14C]PA through the forward reaction of mLPAAT. As shown in Scheme 1, in the presence of unlabeled LPA, the CoA-dependent transacylation reaction occurred (fatty acid transfer from [14C]PA to unlabeled LPA).

We examined the dependence of [14C]LPA formation from [14C]PA as a function of CoA in the presence of unlabeled LPA (Fig. 3B). The [14C]LPA formation was dependent on the concentration of CoA, the apparent K_m value being −5 μM (Fig. 3B).

Next, we tested 1-[14C]palmitoyl-2-linoleoyl-PA or 1-palmityl-2-[14C]linoleoyl-PA as a substrate to define the positional specificity of cleavage of the fatty acyl moiety (Fig. 3C). [14C]LPA was formed from 1-[14C]palmitoyl-2-linoleoyl-PA but not from 1-palmityl-2-[14C]linoleoyl-PA, indicating that cleavage of the fatty acyl moiety was specific for the sn-2 position of PA.

We tested various molecular species of PA as substrates for [14C]LPA formation (Fig. 3D). 1-[14C]Palmityl-2-myristoyl-PA, 1-[14C]palmitoyl-2-palmitoyl-PA, and 1-[14C]palmitoyl-2-linoleoyl-PA served as the best substrates. There seemed to be a correlation between the fatty acyl moiety at the sn-2 position of PA.

**Reverse Reaction of Acyltransferase**

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**Fig. 1. Acyl-CoA acyltransferase activity and substrate specificity of recombinant mLPAAT expressed in JC201 cells.** A, expression of mLPAAT in E. coli. Membrane fractions from JC201 cells (lane 1) and JC201 cells harboring pTrc/mLPAAT cultured in the absence (lane 2) or the presence of IPTG (lane 3) were examined by Western blotting using a monoclonal antibody against the His sequence epitope. B, membrane fractions from cells expressing mLPAAT (open circles) or control cells (open diamonds) were incubated with 40 μM LPA (1-acyl-GP) and 30 μM [14C]18:2-CoA for the indicated periods at 30 °C. C and D, membrane fractions from cells expressing mLPAAT were incubated with various lysophospholipids (40 μM) and 30 μM [14C]18:2-CoA (C) or with 40 μM [14C]LPA and 30 μM various acyl-CoAs or free fatty acids (D) for 1 min at 30 °C. After extraction and separation of the phospholipid product, the radioactivity was measured as described under “Experimental Procedures.”

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Independent acyl-CoA synthesis occurred with the membrane fraction from the cells expressing mLPAAT. The membrane fraction was incubated with 25 μM [14C]PA (1-palmityl-2-[14C]linoleoyl) and 300 μM CoA in the presence of 1% bovine serum albumin. After extraction of acyl-CoA, the extract was separated by TLC. As shown in Fig. 2A, a radioactive band corresponding to standard acyl-CoA was detected (lane 2), suggesting that [14C]linoleoyl-CoA was formed from 2-[14C]linoleoyl-PA. In the absence of CoA, this radioactive band was not detected (lane 1). There was no activity in the membrane fraction from mock-transfected cells even in the presence of 1 mM CoA, confirming that the acyl-CoA synthetic activity was due to recombinant mLPAAT (Fig. 2B, open circle).

mLPAAT-catalyzed [14C]linoleoyl-CoA synthesis was CoA-dependent (Fig. 2B, closed circles). The apparent K_m value for CoA on acyl-CoA formation was 100 μM (Fig. 2B, inset). Desulfur-CoA and dephospho-CoA did not serve as substrates for acyl-CoA synthetase (Fig. 2C).

We examined the requirement of ATP for the acyl-CoA synthesis because acyl-CoA is a high energy form of fatty acid (Fig. 2C). The addition of ATP-MgCl_2 did not significantly affect acyl-CoA synthesis from 2-[14C]linoleoyl-PA. Treatment with apyrase (0.5 units/ml with 1 mM MgCl_2) to deplete residual ATP also did not affect acyl-CoA formation. These results indicate that the [14C]-labeled acyl-CoA was synthesized through an ATP-independent process and that acyl-CoA synthetase was not involved in the process.
ward LPA was due to the recombinant mLPAAT.

The effects of various phospholipids on the CoA-dependent transacylation activity toward LPA were examined. None of the phospholipids other than PA caused a significant increase in transacylation above that seen with CoA alone (Fig. 4). These results demonstrated a donor specificity for PA. Various molecular species of PA were able to serve as acyl donors (data not shown). The donor specificities of acyl-CoA formation (Fig. 2D) and transacylation activity (Fig. 4) were similar, consistent with ATP-independent acyl-CoA synthesis through the reverse reaction of mLPAAT and subsequent acylation of LPA with newly synthesized acyl-CoA through the forward reaction of LPAAT (Scheme 1).

DISCUSSION

Long chain fatty acyl-CoA is a metabolically active form of fatty acid, and various modes of enzymatic conversion of fatty acids, including desaturation, chain elongation, and oxidation, occur mainly in the form of fatty acyl-CoA in mammalian
tissues (33). Another important role of acyl-CoA is as an acyl donor in the formation of various types of simple and complex lipid molecules (33). In addition to its role as a metabolic intermediate, long chain fatty acyl-CoA is known to modulate various enzyme and cell functions. Roles for acyl-CoA in the modulation of the activities of enzymes such as Na\(^+\), K\(^-\)-ATPase (34), Ca\(^{2+}\)-ATPase (35), and protein kinase C (36) and in the function of nuclear hormone receptors (37, 38) have been proposed. It has also been demonstrated that acyl-CoA is required for the budding of transport vesicles from Golgi cisterna (39). Protein acylation involving acyl-CoA has also been reported to contribute significantly to the regulation of protein function (40, 41).

As for the biosynthesis of acyl-CoA, long chain acyl-CoA synthetases are known to catalyze the ligation reaction of free fatty acid and CoA with the consumption of ATP (33, 42). Very long chain acyl-CoA synthetases catalyze similar reactions, but with a preference for very long chain fatty acids (43). These synthetase enzymes have been purified, and their cDNAs have been isolated (42, 43). These acyl-CoA synthetases possess a conserved ATP-binding site and comprise a gene family.

Previously we found another acyl-CoA synthetic pathway involving ATP-independent and CoA-dependent synthesis of acyl-CoA in microsomal fractions of mammalian tissues (15, 19, 20). Substantial acyl-CoA was produced when microsomes were incubated with CoA in the absence of ATP-Mg\(^{2+}\). We considered that this acyl-CoA synthetic activity may be due to the reverse reaction of acyl-CoA:lysophospholipid acyltransferases, because the generation of lysophospholipids, such as LPC and LPI, occurred (15, 19, 20). This idea was also proposed by other investigators, but no conclusive supporting evidence was presented (9, 10, 18). Generally, the biochemical characterization of enzymes involved in phospholipid metabolism has been dif-

![Fig. 3. CoA-dependent LPA formation from PA by mLPAAT. A, membrane fractions from mock-transfected (open columns) or mLPAAT-transfected cells (filled columns) were incubated with 50 μM \(^{[14]C}\)PA (U-\(^{14}C\)glycerol-labeled di-palmitoyl-PA) in the absence (left pair of columns) or presence of 50 μM CoA (center pair of columns), or with 50 μM CoA plus 40 μM unlabeled LPA (right pair of columns) for 30 min at 30 °C. B, membrane fractions from cells expressing mLPAAT were incubated with 50 μM \(^{[14]C}\)PA and 40 μM unlabeled LPA in the presence of the indicated concentrations of CoA for 30 min at 30 °C. The radioactivity of LPA was measured after extraction and purification. Inset, double reciprocal plots of the data in Fig. 3B. C, membrane fractions from cells expressing mLPAAT were incubated with 50 μM \(^{[14]C}\)palmitoyl-2-linoleoyl-PA (left panel) or 1-palmitoyl-2-[\(^{14}C\)cholesterol palmitoyl-PA (right panel) in the absence or the presence of 50 μM CoA plus 40 μM unlabeled LPA for 30 min at 30 °C. The radioactivity of LPA (filled columns) or free fatty acid (open columns) was measured after extraction and purification. D, various PA species (1-[\(^{14}C\])palmitoyl-2-myristoyl-PA, 1-[\(^{14}C\])palmitoyl-2-palmitoyl-PA, 1-[\(^{14}C\])palmitoyl-2-stearoyl-PA, 1-[\(^{14}C\])palmitoyl-2-linoleoyl-PA, 1-[\(^{14}C\])palmitoyl-2-oleoyl-PA, and 1-[\(^{14}C\])palmitoyl-2-arachidonoyl-PA) were used as substrates. Membrane fractions from cells expressing mLPAAT were incubated with PA species in the absence (open columns) or the presence of 50 μM CoA plus 40 μM unlabeled LPA (filled columns) for 30 min at 30 °C. The radioactivity in the product (LPA) was measured after extraction and purification.

![Fig. 4. CoA-dependent transacylation activity toward \(^{[14]C}\)LPA in membrane fractions from cells expressing mLPAAT. Membrane fractions from cells expressing mLPAAT were incubated with 40 μM \(^{[14]C}\)LPA in the absence (open column) or the presence of 50 μM CoA and various phospholipids (100 μM in shaded columns and 1 mM in filled columns) as acyl donor lipids for 30 min at 30 °C. The radioactivity in PA was measured as described under “Experimental Procedures.”](http://www.jbc.org/)}
ficult, because: 1) most of the enzymes are membrane-bound; 2) the substrates are usually water-insoluble and are present in membrane preparations, which prevents accurate kinetic studies; and 3) the enzymes are often denatured during solubilization and purification. In the case of acyl-CoA:lysophospholipid acyltransferases, precise biochemical study of solubilized and purified enzymes has not been successful.

The present results for LPAAT gene-transfected cells revealed a mechanism for ATP-independent acyl-CoA formation from PA. Several lines of evidence indicate that this acyl-CoA synthetic route is due to the reverse reaction of LPAAT: 1) ATP-independent acyl-CoA synthesis occurred in membrane fractions containing mLPAAT but not in control membranes (Fig. 2, A and B); 2) The reaction is specific for PA as an acyl donor (Fig. 2D); and 3) LPA is formed during the acyl-CoA synthesis (Fig. 3). This is the first direct and conclusive evidence for an acyl-CoA synthesizing enzyme other than acyl-CoA synthetase, and it also reveals a novel function for LPAAT.

The CoA-dependent transacylation system catalyzes the transfer of fatty acids esterified in phospholipids to lysophospholipids in a CoA-dependent manner without the generation of free fatty acids. The results of the present study have led to the hypothesis that the CoA-dependent transacylation comprises the combination of the reverse and forward reactions of acyl-CoA:lysophospholipid acyltransferases, as depicted in Scheme 1. The acylation activity shows strict specificity for PA as an acyl donor and for LPA as an acyl acceptor.

Although we found here that the α isoform of LPAAT catalyzed the transacylation reaction using recombinant enzymes, we did not examine whether or not another isoform (β form) can catalyze the transacylation reaction. However, we found CoA-dependent transacylation activity toward LPA in rat liver microsomes. In addition, ATP-independent, CoA and PA-dependent acyl-CoA synthetic activity was found in liver microsomes.2 These results suggested that similar enzymatic reactions to those observed here occur in mammalian tissues. Because the β isoform is predominant in liver (23, 25), rat LPAAT-β would be also involved in the transacylation reaction.

The question of whether or not the reaction in Scheme 1 applies to other acyl-CoA:lysophospholipid acyltransferases besides LPAAT needs to be considered. The ATP-independent acyl-CoA synthetic activity observed in microsomal fractions from mammalian tissues resembles the acyl-CoA formation by mLPAAT in the following aspects: 1) acyl-CoA is formed through an ATP-independent process (15, 19, 20); 2) phospholipids probably act as acyl donors, because lysophospholipids such as LPC and LPI are formed during the acyl-CoA synthesis (19); and 3) partially purified acyl-CoA:LPI acyltransferase from rat liver microsomes exhibits ATP-independent acyl-CoA synthetic activity from PI.2 These similarities suggest that the ATP-independent acyl-CoA synthesis and concomitant generation of lysophospholipids observed in microsomes (19) are due to the reverse reactions of acyl-CoA:lysophospholipid acyltransferases, in particular, acyl-CoA:LPC acyltransferase and acyl-CoA:LPI acyltransferase. The acyl groups of a variety of phospholipid donors could thus feed into the common acyl-CoA pool, which would be available to furnish acyl groups to a variety of lysophospholipid acceptors. In this process, CoA-dependent transacylation could occur between phospholipids and lysophospholipids with different polar head groups (Scheme 1). The acyl exchange reactions occurring because of the reverse and forward reactions of multiple acyltransferases could be expected to contribute to fatty acid remodeling of phospholipids. It is worth noting that such remodeling could occur without the consumption of ATP.

The extent of ATP-independent acyl-CoA synthesis is potentially significant. In rat liver microsomes, 25% of PI was converted to LPI during 30 min of incubation in a CoA-dependent/ATP-independent manner (19). Approximately 5% of PC was converted to LPC under the same conditions. These activities were probably catalyzed by the reverse reaction of the acyl-CoA:LPC acyltransferase and acyl-CoA:LPI acyltransferase enzymes, respectively. These activities are likely to be underestimated because the measured activities reflect the net of the reverse and forward reactions of the acyltransferase, as depicted in the first equation in Scheme 1. The apparent $K_m$ values for CoA in the acyl-CoA synthesis from PA (100 μM in Fig. 2) and from liver microsomal phospholipids (180 μM in Ref.

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2 A. Yamashita, unpublished observation.
C, protein, and lipid kinases, mobilize Ca2+ following various agonists (47). PA species can stimulate phospholipase C in an intracellular messenger in a signaling pathway activated by a surface agonist (46). On the other hand, PA is known to be a key regulatory lipid in multiple, specific G protein-coupled receptors on the cell membrane, which may be involved in cytoskeleton remodeling, and neurite retraction (44–46). LPA is also recognized as a bioactive lipid, which may be a prototype for other microsomal CoA-dependent transacylation systems specific for LPC and LPI.

Both the substrate (LPA) and product (PA) of LPAAT are involved in multiple cellular processes with roles in mitogenesis, tumor cell growth and invasion, proliferation and activation of immune cells, actin cytoskeleton remodeling, and neurite retraction (44–46). LPA is released by activated cells to influence target cells by acting on multiple, specific G protein-coupled receptors on the cell surface (46). On the other hand, PA is known to be a key intracellular messenger in a signaling pathway activated by various agonists (47). PA species can stimulate phospholipase C, protein, and lipid kinases, mobilize Ca2+, and activate NADPH oxidase and gene transcription. Because LPAAT can catalyze the interconversion of LPA and PA, it may be involved in fine tuning of the signaling actions of LPA and PA. Recently, LPAAT inhibitors were shown to block the inflammatory response produced by an increase in PA (48). West et al. (23) found that overexpression of LPAAT and the resulting increase in LPAAT activity were correlated with enhanced expression and synthesis of tumor necrosis factor-α and interleukin-6 in cells treated with interleukin-1β. Identification of the LPAAT isozyme involved in modulation of the inflammatory response will be crucial for the design of therapeutic agents.

In conclusion, we have found an ATP-independent biosynthetic pathway for acyl-CoA, which is based on the reverse reaction of LPAAT. The combination of the reverse and forward reactions of LPAAT constitute a CoA-dependent transacylation system for LPA, which may be a prototype for other microsomal CoA-dependent transacylation systems specific for LPC and LPI.

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