J. Biochem. **122**, 1-16 (1997)

Acyltransferases and Transacylases Involved in Fatty Acid Remodeling of Phospholipids and Metabolism of Bioactive Lipids in Mammalian Cells

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Received for publication, April 1, 1997

Over 100 different phospholipid molecular species are known to be present in mammalian cells and tissues. Fatty acid remodeling systems for phospholipids including acyl-CoA: lysophospholipid acyltransferases, CoA-dependent and CoA-independent transacylation systems and lysophospholipase/transacylase are involved in the biosynthesis of these molecular species. Acyl-CoA:1-acyl-2-lysophospholipid acyltransferase prefers polyunsaturated fatty acyl-CoAs as acyl donors while acyl-CoA:2-acyl-1-lysophospholipid acyltransferase prefers saturated fatty acyl-CoAs. Therefore, the acyl-CoA:lysophospholipid acyltransferase system is involved in the synthesis of the phospholipid molecular species containing *sn*-1 saturated and *sn*-2 unsaturated fatty acids. The CoA-dependent transacylation system catalyzes the transfer of fatty acids esterified in phospholipids to lysophospholipids in the presence of CoA without the generation of free fatty acids. The CoA-dependent transacylation reaction in rat liver exhibits strict fatty acid specificity, *i.e.*, three types of fatty acids (20:4, 18:2, and 18:0) are transferred. On the other hand, the CoA-independent transacylase catalyzes the transfer of C20 and C22 polyunsaturated fatty acids from diacyl phospholipids to various lysophospholipids, in particular, ether-containing lysophospholipids, in the absence of any cofactors. The CoA-independent transacylase is assumed to be involved in the accumulation of polyunsaturated fatty acids in ether-containing phospholipids and in the removal of deleterious ether-containing lysophospholipids. These acyltransferases and transacylases are involved in not only the remodeling of fatty acids but also the synthesis and degradation of some bioactive lipids and their precursors. In this review, the properties of these fatty acid remodeling systems and their possible roles in the biosynthesis of bioactive lipids are described.

Key words: acyl-CoA, acyltransferase, arachidonic acid, fatty acid remodeling, lysophospholipid.

Various kinds of fatty acids are distributed in phospholipids in various mammalian tissues (*1-8*). The fatty acyl residues of individual phospholipids appear to be under strict metabolic regulation. In general, saturated fatty acids are esterified at the *sn*-1 position while unsaturated fatty acids, such as arachidonic acid, are commonly found at the *sn*-2 position. It is well known that C20 polyunsaturated fatty acids such as arachidonic acid are not usually introduced into phospholipids through *de novo* synthesis. Arachidonic acid is incorporated into phospholipids during the fatty acid remodeling of phospholipids. In this review, we summarize and discuss up-to-date information regarding acyltransferases and transacylases involved in the fatty acid remodeling of phospholipids. Furthermore, some types of bioactive lipids are known to be derived from phospholipids (*9, 10*). Phospholipases, acyltransferases and transacylases are known to be involved in the synthesis and degradation of bioactive lipids and their precursor molecule. We also refer to the possible roles of acyltransferases and transacylases in the biosynthesis of bioactive lipids, in particular, platelet-activating factor (PAF), *N*-arachidonylethanolamine (anandamide), long chain fatty acyl-CoA, and lysophospholipids.

1. Compositions of phospholipids

Various types of phospholipid classes with different polar head groups are present in mammalian cells and tissues, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS). Each phospholipid is subclassified, diacyl, alkylacyl or alkenylacyl, according to differences in the chemical linkage of the fatty chain at the *sn*-1 position of the glycerol backbone, such as acyl, ether and vinyl-ether bonds (*2-4*). Furthermore, the structure of glycerophospholipids exhibits a high degree of heterogeneity with

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Abbreviations: CGP, choline glycerophospholipid; CoA, coenzyme A; EGP, ethanolamine glycerophospholipid; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; GPI, glycerophosphoinositol; IGP, inositol glycerophospholipid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SGP, serine glycerophospholipid; fatty acids are designated in terms of the number of carbon atoms : the number of double bonds, *e.g.* 20:4 denotes arachidonic acid.

respect to molecular species, which results from the combination of the structures of the fatty chains at the *sn*-1 and *sn*-2 positions. Therefore, over 100 phospholipid species with different structures are present in mammalian cells and tissues. Several methods have been developed for separation of the molecular species of glycerophospholipids by HPLC (*11-16*). These methods have enabled the precise analysis of the molecular species of glycerophospholipids. The distributions of molecular species of individual phospholipids differ among various cells and tissues. The molecular species of diacyl, alkylacyl and alkenylacyl glycerophospholipids of rabbit alveolar macrophages and those of diacyl species of rat liver are shown in Tables I and II.

Substantial amounts of ether-containing phospholipids such as the alkylacyl subclass of CGP and the alkenyl subclass of EGP are present in various tissues [reviewed by Sugiura and Waku (*4*)]. In particular, high levels of alkylacyl-GPC are present in white blood cells such as macrophages and polymorphonuclear leukocytes. In rabbit alveolar macrophages, alkylacyl-GPC accounts for 32.5% of CGP and 11.3% of total phospholipids (*4, 15*). The high amount of alkylacyl-GPC in these cells is important in view of the formation of platelet-activating factor through the remodeling pathway (deacylation-acetylation) (*4, 7*). Alkenylacyl-GPE (plasmalogen) is widely distributed in various tissues, but not liver. It is well known that nervous tissues are rich in alkenylacyl-GPE. About one-half to two-thirds of EGP is accounted for by the alkenylacyl type in the whole brain. The biological role of alkenylacyl-GPE in nervous tissues remains obscure and speculative. However, it seems obvious that this phospholipid is important in nervous tissues, since alkenylacyl-GPE is very rapidly catabolized during the course of demyelinating diseases and ischemia in the brain (*17*). The alkenylacyl subclass of CGP (choline plasmalogen) is present in the heart and spermatozoa.

Arachidonic acid (20:4), a common precursor for a variety of biologically active chemical mediators, is generally esterified at the *sn*-2 position of each class of phospholipid. It should be noted that the levels of 20:4 in ether-linked phospholipids are higher than those in the diacyl counterparts. In rabbit alveolar macrophages, a large portion of 20:4 is located at the *sn*-2 position of alkylacyl species of CGP and alkenylacyl species of EGP.

The liver is a poor source of ether-containing phospholipids. Only trace amounts are found in the liver of various mammalian species (*4*). The major portions of CGP and EGP are diacyl species. In rat liver, 20:4-containing molecular species of diacyl-GPC and diacyl-GPE account for 44 and 35% of diacyl-GPC and diacyl-GPE, respectively (16:0-20:4 and 18:0-20:4 species of CGP and EGP) (Table II).

In contrast to CGP and EGP, the majority of IGP and SGP is of the diacyl type in various mammalian tissues and cells, an exception being the PI moiety of glycosyl phosphoinositol (GPI) anchor proteins (*18*). The molecular species compositions of IGP and SGP have characteristic features. The predominant molecular species comprising diacyl-GPI is the 18:0-20:4 species, which represents 40 and 58% of IGP in rabbit alveolar macrophages and rat liver, respectively. The majority of SGP comprises 18:0-containing species such as 18:0-18:1, 18:0-20:4, and 18:0-22:6. In alveolar macrophages, 18:0-18:1 species account for 64% of SGP. In rat liver, 18:0-20:4 and 18:0-22:6 species account for a large portion of SGP.

TABLE I. **Distribution of molecular species of diacyl, alkylacyl, and alkenylacyl glycerophospholipids in alveolar macrophages.**

Molecular species[a]	CGP		EGP		PI	PS
	Diacyl	Alkylacyl	Diacyl	Alkenylacyl		
	(%)					
18:2-20:4	0.9	1.2	0.4		0.1	
16:0-22:6	0.4	1.9	0.3	2.7	0.1	
18:2-18:2	2.8	0.5	0.6		0.2	
18:1-18:3	0.4		0.2			
16:0-18:3	1.8	0.9	0.3			
18:1-22:5(*n*-3)		1.5	0.1			
18:1-20:4	1.8	8.0	2.6	3.9	1.6	0.4
16:0-22:5(*n*-3)	1.5	6.9	1.2	11.1		
16:0-20:4	6.7	38.7	3.0	48.5	3.3	0.6
16:0-22:5(*n*-6)	0.2	0.8	0.4	1.3		
18:1-18:2	5.1	2.7	4.0	0.3	2.8	1.0
18:0-22:6			0.4	0.5	0.2	0.6
16:0-18:2	7.0	13.5	3.4	3.5	2.6	1.2
18:1-22:4		0.3	0.1	0.2	0.2	
18:1-20:3		1.1	0.2		0.4	0.1
16:0-22:4	0.4	2.1	0.5	2.4		0.4
18:0-18:3	0.7		0.7			
18:0-22:5(*n*-3)	0.5	0.7	3.4	3.3		0.5
18:0-20:4	1.8	3.6	13.9	10.7	39.7	4.5
18:0-22:5(*n*-6)			0.5	0.2		0.9
18:1-18:1	2.1	0.8	3.5	0.2	5.2	2.4
16:0-18:1	21.4	4.2	5.6	8.9	6.6	2.3
18:0-18:2	15.5	1.3	18.7	1.0	12.7	9.6
18:0-20:3			0.9	0.1	2.5	1.8
16:0-16:0	17.0	4.7	1.1	0.1	0.7	0.7
18:0-22:4			1.4	0.7	0.8	1.8
18:0-20:3			0.2		0.7	
18:0-18:1	8.2	0.9	29.1	1.9	14.1	64.4
18:0-16:0	4.4	1.2	0.7	0.3	0.7	1.3

[a]The molecular species of each phospholipid were separated by reverse phase-HPLC after the conversion to 1,2-diacyl-3-acetylglycerol derivatives (Ref. *15*).

TABLE II. **Molecular species compositions of glycerophospholipids in rat liver.**

Molecular species	Diacyl-GPC[a]	PC[b]	PE[b]	PI[b]	PS[b]
	(%)				
18:2-20:4	1.3				
18:1-22:6	0.8				
16:0-22:6	4.6	3.9	9.0		
18:2-18:2	0.9	1.5			
18:1-20:4	4.5				
16:0-20:4	22.0	9.7	10.7		
18:1-18:2	3.9	3.8	6.1		
16:0-18:2	11.6	14.4	12.2		
16:0-20:3(*n*-6)	0.8	1.3			
16:0-20:3(*n*-9)	nd	1.5			
18:0-20:4	22.2	16.1	24.5	57.8	39.5
18:1-18:1	0.6	6.4	2.2		
16:0-18:1	8.6	1.4	1.2		
18:0-18:2	13.8	14.8	6.8		5.0
18:0-20:3(*n*-6)	0.7	1.6			
18:0-20:3(*n*-9)	nd				
18:0-18:1	1.7	3.4	2.9	5.8	5.5
18:0-22:6		3.6	7.7	3.1	24.8

[a]The molecular species of diacyl-GPC in rat liver microsomes were separated by reverse phase-HPLC after the conversion to 1,2-diacyl-3-acetylglycerol derivatives (Ref. *16*). [b]The molecular species of PC, PE, PI, and PS in rat liver were separated by reverse phase-HPLC without any modification (Ref. *11*).

2. Biosynthesis of phospholipid molecular species *via de novo* pathway

The biosynthesis of individual molecular species of phospholipids appears to be under strict metabolic regulation. The primary pathways involved in the incorporation of fatty acids into the glycerol backbone of diacylglycerophospholipids are the acyl-CoA:glycerol-3-phosphate (GP) acyltransferase and acyl-CoA:1-acyl-GP acyltransferase systems for *de novo* synthesis (*1, 2*). Phosphatidic acid (diacyl-GP) in rat liver is known to contain a preponderance of monoenoic and dienoic species, lesser amounts of polyenoic species being present (*19*). The fatty acid specificity of the *de novo* synthesis of phosphatidic acid in rat liver *in vivo* or in rat liver slices demonstrated that *sn*-1 saturated *sn*-2 mono- and dienoic species were predominant (*19-22*). Okuyama and Lands (*23*) suggested that the selectivity of acyl-CoA:1-acyl-GP acyltransferase in an *in vitro* assay involving rat liver microsomes is dependent on the concentration of 1-acyl-GP. The selectivity of acylation of 1-acyl-GP with various acyl-CoAs strictly resembles the fatty acid specificity of *in vivo* phosphatidic acid synthesis when the concentration of 1-acyl-GP is low, whereas relatively nonselective acylation occurs with a high 1-acyl-GP concentration. These results show the strict fatty acid specificity of *de novo* acyltransferase systems.

In the case of ether-containing phospholipids, 1-alkyl-2-acyl-GP, the alkyl counterpart of phosphatidic acid, is synthesized through the following sequential reactions; acylation of dihydroxyacetone phosphate, exchange of the 1-acyl-group with a 1-alkyl-group, reduction to form 1-alkyl-GP, and acylation of 1-alkyl-GP by acyl-CoA:1-alkyl-GP acyltransferase (*2, 3*). Fleming and Hajra (*24*) reported that rat brain acyl-CoA:1-alkyl-GP acyltransferase selected polyunsaturated acyl-CoAs such as 20:4-CoA and 22:6-CoA over saturated species with a low concentration of 1-alkyl-GP. This acyltransferase could partially determine the composition of acyl groups in ether-containing phospholipids.

The utilization of phosphatidic acid involves one of two principal reactions; its dephosphorylation to diacylglycerol or its activation to CDP-diacylglycerol. PC and PE are synthesized from diacylglycerol through the CDP-choline and CDP-ethanolamine pathways, while PI is synthesized from CDP-diacylglycerol and inositol (*2*). Therefore, the molecular species composition of newly synthesized phospholipids seems to reflect more or less that of phosphatidic acid. The molecular species composition of newly synthesized PC in rat liver *in vivo* or in rat liver slices demonstrated that mono- and dienoic species were predominant (*19-22*). In rabbit alveolar macrophage microsomes, the profiles of newly synthesized phosphatidic acid and CDP-diacylglycerol species were similar to those of newly formed PI species (*25*), suggesting that the enzymes involved in the formation of PI species from phosphatidic acid show little specificity for different molecular species of substrates.

In the case of the molecular species composition of newly synthesized PE, hexaenoic species are relatively abundant compared to in the case of PC (*19, 20, 22*). Noteworthy is the conspicuous absence of disaturated species in either the newly synthesized diacyl-GPE or the pre-existing diacyl-GPE, in contrast to the presence of disaturated species of diacyl-GPC (*20*). It has been demonstrated that CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase (*26, 27*) and PE *N*-methyltransferase (*22*) both show selectivity for docosahexaenoic acid-containing species. Masuzawa *et al.* (*28, 29*), Nakagawa and Waku (*30*), and Onuma *et al.* (*31*) demonstrated that ethanolaminephosphotransferase may also show selectivity for docosahexaenoic acid-containing alkylacylglycerols. These results show that docosahexaenoic acid-containing phospholipids are mainly synthesized *de novo*.

3. Fatty acid remodeling system

The molecular species of phospholipids present in various tissues and cells are considerably different from those of their precursor molecule, phosphatidic acid. These results suggested that the fatty acid remodeling of phospholipids occurs after *de novo* synthesis. It has been widely accepted that arachidonic acid is introduced into phospholipids mainly through the remodeling pathway. Similarly, it has been suggested that a large portion of stearic acid is also incorporated into phospholipids through remodeling. The reactions and properties of acyltransferases and transacylases involved in remodeling are summarized in Fig. 1, A-D, and Table III.

3.1. Acyl-CoA:lysophospholipid acyltransferases. The differences in the turnover rates of the fatty acyl and glycerol moieties of phospholipids led Lands (*32, 33*) to the finding of the presence of acyl-CoA:lysophospholipid acyltransferase in rat liver. The enzyme system acylating the *sn*-1 position of 2-acyl-glycerophosphocholine (GPC) has been shown to be more active with saturated acyl-CoAs, whereas the one acylating the *sn*-2 position of 1-acyl-GPC is more active with unsaturated acyl-CoAs (*34*). These observations emphasized the importance of these enzyme systems in the selective placement of different fatty acids between the two positions of glycerophospholipids. Acyl-CoA:lysophospholipid acyltransferase activities are widely distributed in various cells and tissues, and are tightly bound to microsomal and plasma membranes.

Numerous studies have been conducted on the nature of acyltransferases, such as their acyl-CoA substrate specificity. Kanoh and Ohno (*35*) demonstrated that 1-acyl-GPC is effectively taken up by rat liver, and is acylated *in vivo* by fatty acids with tetraene predominantly and ones with diene to a lesser extent, but very little by ones with monoene. However, the selectivity observed *in vivo* is apparently inconsistent with the specificity observed *in vitro*, since the microsomal acyl-CoA:1-acyl-GPC acyltransferase system utilizes oleoyl-, linoleoyl-, and arachidonoyl-CoAs at comparable rates. Okuyama *et al.* (*36*) demonstrated that more arachidonoic acid was incorporated than oleic acid when very low concentrations of acceptor 1-acyl-GPC were added to an *in vitro* assay system involving rat liver microsomes, although the maximal velocities for oleoyl-CoA and arachidonoyl-CoA were approximately the same. Thus, the higher selectivity for arachidonic acid observed in rat liver *in vivo* could be correlated with the relatively higher affinity in this reaction for 1-acyl-GPC observed *in vitro*, which is seen at low concentrations of the acceptor. Lands *et al.* (*37*) determined the acyl-CoA fatty acid specificity of acyl-CoA:1-acyl-GPC acyltransferase in rat liver microsomes, measuring the competitive effectiveness defined as the equivalent concentration of acyl-CoA competitively incorporated into 1-acyl-GPC *versus* arachidonoyl-CoA. This

assay revealed that 20:4(n-6)-CoA, 18:3(n-3)-CoA, 18:3(n-6)-CoA, and 20:5(n-3)-CoA were the most preferential substrates for the acyltransferase, while the saturated fatty acyl-CoAs were not. The acyl-CoA selectivity of acyl-CoA:2-acyl-GPC acyltransferase was also examined (*1, 34*). The relative rates of incorporation at the *sn*-1

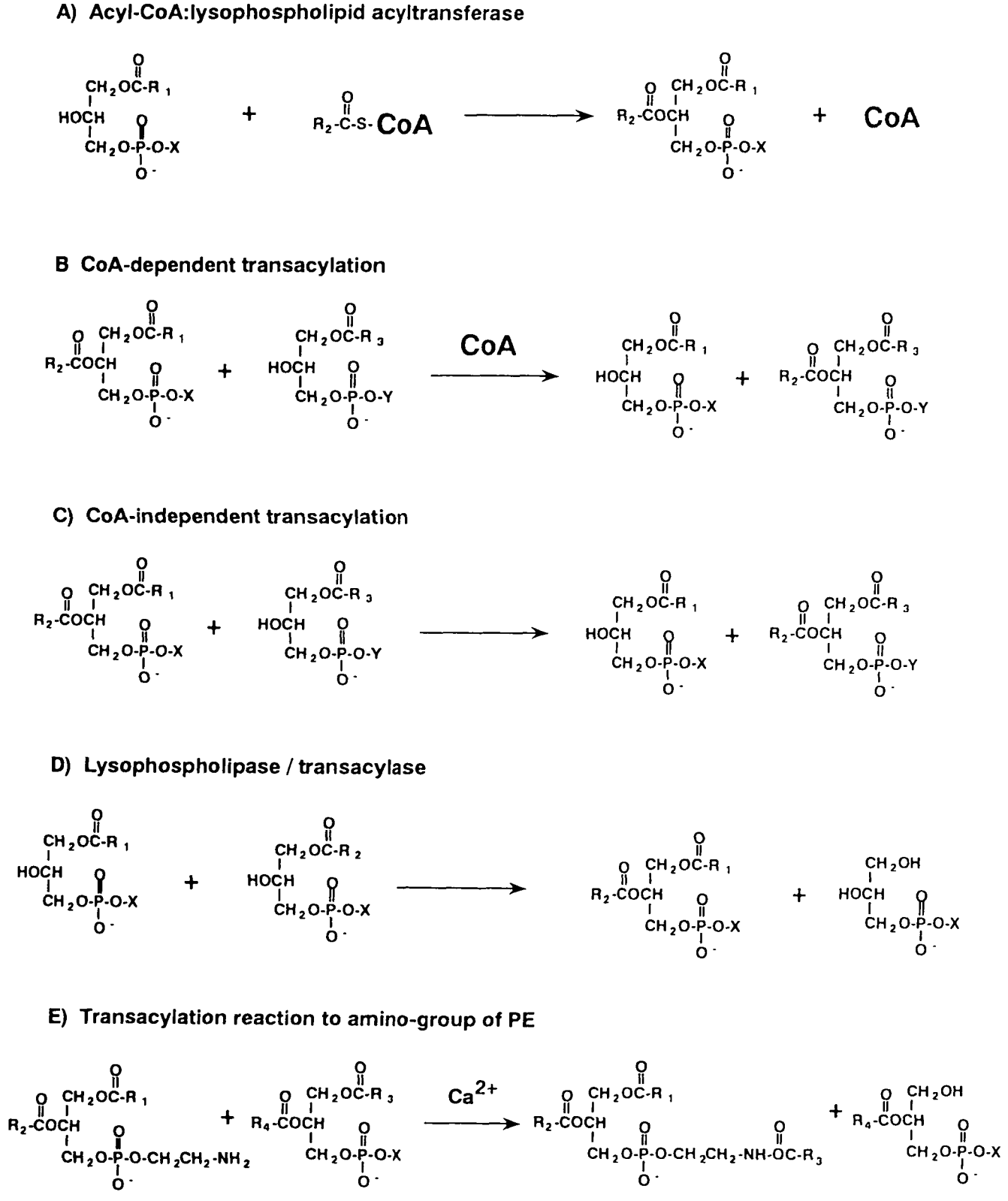

Fig. 1. **Acyltransferase and transacylase reactions involved in the fatty acid remodeling of phospholipids and the synthesis of bioactive lipids.**

position of PC were in the following order; 18:0=16:0>14:0=12:0>18:1, suggesting that saturated fatty acids were incorporated at the *sn*-1 position, while unsaturated fatty acids were less effective. From these acyl-CoA specificities, acyl-CoA:1-acyl-GPC acyltransferase was thought to be involved in the incorporation of polyunsaturated fatty acids, in particular arachidonic acid, at the *sn*-2 position of PC, whereas acyl-CoA:2-acyl-GPC acyltransferase was involved in the incorporation of saturated fatty acids at the *sn*-1 position of this phospholipid. These enzymes are presumably coupled with phospholipase A, which generates lysophospholipids (deacylation-reacylation cycle, Lands cycle, Fig. 2).

Acylation activity of lysophosphatidylinositol is also present in various tissues and cells (*38, 39*). Acyl-CoA:1-acyl-GPI acyltransferase also prefers polyunsaturated fatty acyl-CoAs (*38, 39*). Interestingly, *n*-6 fatty acids are relatively more preferred substrates than *n*-3 fatty acids for the acyl-CoA:1-acyl-GPI acyltransferase, as compared with acyl-CoA:1-acyl-GPC acyltransferase (*39*). This enzyme is assumed to be implicated in the production and accumulation of 20:4-containing species of PI.

The conversion of 1-acyl-GPS into molecular species of PS using endogenous fatty acids in rat liver homogenates occurred in the presence of ATP and CoA, and most PS formed was the tetraenoic species (81%) (*40*). These results obtained with endogenous acyl donors suggest that such a mechanism may partly account for the enrichment of arachidonic acid in liver PS. On the other hand, acyl-CoA:2-acyl-GPS acyltransferase exhibits a preference for long-chain saturated over unsaturated acyl-CoAs, and the highest acylation rates were observed with stearoyl-CoA, indicating that such an enzyme specificity partly accounts for the abundance of stearic acid at the *sn*-1 position of PS (*41*).

Therefore, the primary physiological role of acyl-CoA:lysophospholipid acyltransferases is to provide phospholipids having a saturated fatty acid at the *sn*-1 position and an unsaturated fatty acid at the *sn*-2 position.

On the other hand, acyl-CoA:1-acyl-GPC acyltransferase in adult rat lung alveolar type II epithelial cells was found to prefer palmitoyl-CoA over oleoyl-CoA, indicating that this acyltransferase is involved in the synthesis of dipalmitoyl-GPC, the major active component of the pulmonary surfactant, in this tissue (*42*).

Acyl-CoA:1-alkenyl-GPC acyltransferase activity is absent in rat liver microsomes (*43*). However, Waku and Lands (*44*) demonstrated that acyl-CoA:1-alkenyl-GPC

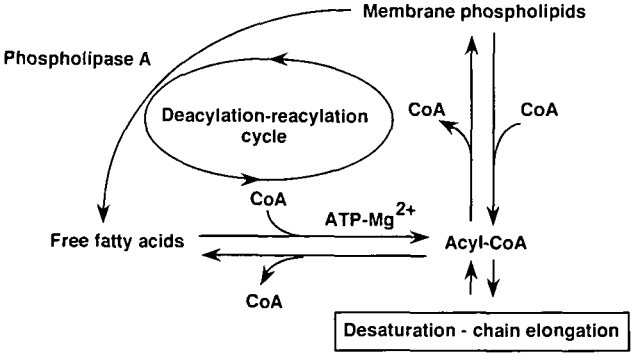

Fig. 2. **Deacylation-reacylation cycle.**

TABLE III. **Properties of acyltransferases and transacylases.**

	Cofactor	Acyl donor	Acyl acceptor	Specificity of fatty acid transferred	References
Acyl-CoA:lysophospholipid acyltransferase		Acyl-CoA	1-Acyl-GPC (150 μM)	20:4≥18:2=18:1≫16:0≥18:0	*36*
			1-Acyl-GPC (12.5 μM)	20:4≫18:1 (Rat liver microsomes)	*36*
			1-Acyl-GPC (89 μM)	18:3(*n*-6)=18:3(*n*-3)=20:5(*n*-3)>20:4>20:3(*n*-3)>18:2≫18:1≫16:0=18:0 (competitive effectiveness) (Rat liver microsomes)	*37*
			2-Acyl-GPC	18:0≥16:0>14:0>18:1 (Rat liver microsomes)	*34*
			1-Acyl-GPI	20:4≫18:2>18:1≥16:0>18:0 (Rat brain microsomes)	*38*
			1-Acyl-GPI	20:4>18:2>18:3(*n*-3)≥20:5(*n*-3)=20:3(*n*-6)≫20:2(*n*-6)=18:1(*n*-9)≫16:0>18:0 (Rat platelet microsomes)	*39*
			1-Acyl-GPS	Tetraenes (using endogenous acyl donor)	*40*
				20:4=18:2≫18:1>16:0=18:0 (Rat liver microsomes)	*40*
			2-Acyl-GPS	18:0>16:0>14:0>12:0>18:1≫18:2≫20:4 (Rat liver microsomes)	*41*
CoA-dependent transacylation	CoA	Phospholipid (PI>PC>PE)	1-Acyl-GPC	20:4, 18:2 from *sn*-2 position	*56*
			1-Acyl-GPE	18:0 from *sn*-1 position (Rat liver microsomes)	
			1-Acyl-GPC	20:4, 18:1 from *sn*-2 position	*63*
			1-Acyl-GPE	18:0 from *sn*-1 position (Rat brain microsomes)	
CoA-independent transacylation		Phospholipid	1-Alkyl-GPC 1-Alkenyl-GPE	C20 and C22 polyunsaturated fatty acids from *sn*-2 position	*57*
Lysophospholipase /transacylase		LPC	LPC	16:0>18:0, C>10 fatty acids	*72,73*
Transacylation reaction to amino-group of PE	Ca^{2+}	Fatty acid esterified at *sn*-1 position of phospholipid	Amino residue of PE	16:0, 18:0, 18:1, 18:2 20:4	*119* *128, 130*

acyltransferase activity was present in human erythrocyte membranes and rabbit sarcoplasmic reticulum. Acyl-CoA:1-alkyl-GPC acyltransferase is also present in rabbit sarcoplasmic reticulum (*45*) and Ehrlich ascites tumor cells (*46*). Acyl-CoA:1-alkyl-GPC acyltransferase activity was found in various tissues and cells (*7*). The acyltransferase activities toward 1-alkenyl-GPC and 1-alkyl-GPC are relatively low compared with that toward 1-acyl-GPC. It is unclear at present whether a single enzyme catalyzes the transfer of fatty acids from acyl-CoAs to various acceptors with different chemical linkages at the *sn*-1 position, such as 1-acyl-, 1-alkyl-, and 1-alkenyl-GPC.

Acyl-CoA:lysophospholipid acyltransferases play important roles in regulation of the free arachidonic acid level (*47, 48*). Treatment of cells with stimulatory agents such as ionophore results in enhanced phospholipase A2 activity, and the generation of large amounts of lysophospholipids and fatty acids including arachidonic acid. The released arachidonic acid is converted to various eicosanoids, and excess arachidonic acid is rapidly reacylated through the sequential reactions of acyl-CoA synthetase and acyl-CoA:lysophospholipid acyltransferases. Thus, the activity of acyl-CoA:lysophospholipid acyltransferases determines the level and duration of free arachidonic acid.

There have been several reports concerning the regulation of acyl-CoA:lysophospholipid acyltransferase activity. Treatment of macrophages and platelets with activators of protein kinase C inhibited arachidonoyl-CoA:1-acyl-GPC acyltransferase (*47, 48*). The inhibition of the enzyme activity resulted in an increase in free arachidonic acid and enhanced production of various eicosanoids. In addition, long-term administration of clofibrate and other peroxisome proliferators to rodents induced the acyl-CoA:1-acyl-GPC acyltransferase activity (3-5 times increase compared to in control animals) (*16, 49*). Peroxisome proliferators are known to act *via* "peroxisome proliferator-activated receptors (PPARs)," members of the nuclear receptor superfamily, and to induce various genes of peroxisomal and other organelle-located proteins (*50*). Acyl-CoA:1-acyl-GPC acyltransferase seems to be induced by clofibrate *via* PPAR in a similar manner. Acyltransferase activity in rat submandibular glands is also affected by chronic administration of isoproterenol (*51*).

The molecular nature of acyltransferases has not yet been well characterized. This is due to the difficulty of the solubilization and purification of the enzyme proteins from microsomal membranes. Only three enzymes, acyl-CoA:1-acyl-GPC acyltransferase in bovine brain microsomes (*52*), and acyl-CoA:1-acyl-GPC acyltransferase (*53*) and acyl-CoA:1-acyl-GPI acyltransferase (*54*) in bovine heart microsomes, have been solubilized with the zwitterionic detergent, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and purified to near homogeneity through sequential chromatographies, although cDNA cloning has not been performed. In bovine heart microsomes, the activities of acyl-CoA:1-acyl-GPC acyltransferase and acyl-CoA:1-acyl-GPI acyltransferase have been separated by chromatography (*54*). These enzymes exhibited different molecular masses on SDS-polyacrylamide gel electrophoresis (*53, 54*). In rat liver microsomes, the activities of acyl-CoA:1-acyl-GPC acyltransferase, acyl-CoA:1-acyl-GPI acyltransferase, and acyl-CoA:2-acyl-GPI acyltransferase were separated from each other through several chromatographies (Yamashita *et al.*, manuscript in preparation). This suggested that there are enzymes with different acceptor specificities. In addition, although acyl-CoA:1-acyl-GPC acyltransferase in bovine heart microsomes was effectively solubilized with CHAPS (87% of microsomal activity), the enzyme in rat liver microsomes was solubilized less efficiently (only 30-40% of microsomal activity) by the same procedure (*53*, Yamashita *et al.*, manuscript in preparation). This suggests that the enzymes in different tissues were different from each other. Furthermore, long-term administration of clofibrate to rodents induced acyl-CoA:1-acyl-GPC acyltransferase but not acyl-CoA:2-acyl-GPC acyltransferase activity, suggesting that enzymes responsible for the incorporation at different positions of the glycerol backbone are present (*16, 49*). In any case, it is apparent that various types of acyltransferases exist in mammalian tissues and cells.

3.2. CoA-dependent transacylation reaction. The CoA-dependent transacylation reaction was first reported by Irvine and Dawson (*55*) in rat liver microsomes. This activity is widely distributed in the microsomes of various tissues and cells such as liver (*56*), macrophages (*57-59*), platelets (*60, 61*), brain (*62, 63*), lung (*64*), and testis (*65*). Fatty acids esterified in phospholipids are transferred to lysophospholipids in the presence of CoA without the generation of free fatty acids in the CoA-dependent transacylation reaction (Fig. 1). The K_m of CoA for the CoA-dependent transacylation reaction is very low (1-4 μM). This suggests that the transacylation reaction occurs with physiological concentrations of CoA.

The acyl-donor and acceptor selectivities of the CoA-dependent transacylation reaction have also been thoroughly examined in rabbit liver (*56*), rabbit alveolar macrophages (*57*), and rat brain microsomes (*62, 63*). In rabbit liver microsomes, three types of fatty acids (20:4, 18:2, and 18:0) were transferred to 1-acyl-GPC and 1-acyl-GPE in the presence of CoA, suggesting that this system exhibits strict fatty acid specificity (*56*). 20:4 and 18:2 are transferred from the *sn*-2 position of phospholipids, while 18:0 is transferred from the *sn*-1 position. In brain microsomes, 18:1 is transferred instead of 18:2 *via* the CoA-dependent transacylation system (*62, 63*). Concerning the types of donor phospholipids, CGP, EGP, and IGP were shown to serve as donor phospholipids, diacyl-GPI being the most preferential acyl donor among them.

As for acceptor lysophospholipids, 1-acyl-GPC, 1-acyl-GPE, 1-acyl-GPI, and 1-acyl-GPS serve as effective acceptors. Kramer *et al.* (*60*) found that human platelet membranes exhibited a preference for 1-acyl-GPS as the arachidonic acid acceptor in the CoA-dependent transacylation system. Other lysophospholipids are also utilized as acyl acceptors, albeit at lower rates, in the order of 1-acyl-GPE > 1-alkenyl-GPE > 1-acyl-GPI. In macrophages derived from mouse bone marrow, 1-alkenyl-GPC and 1-alkenyl-GPE are poorer acyl acceptors than the corresponding 1-acyl-lysophospholipids (*59*). The CoA-dependent transacylation system in rat brain also exhibited an acceptor preference, namely 1-acyl-GPC > 1-acyl-GPE $\gg$ 1-alkenyl-GPE (*63*). These results show that ether-containing lysophospholipids are poor acceptors in the CoA-dependent transacylation system.

The remodeling of phosphatidylinositol has been investigated using rat liver microsomes (*66*). Acylation of 2-

acyl-GPI occurs in a CoA-dependent and ATP-independent manner. Stearic acid is the major acyl group esterified at the *sn*-1 position through the CoA-dependent transacylation reaction. Similar CoA-dependent stearic acid transfer from PI to phosphatidic acid was observed in bovine testis microsomes (*65*).

As for the mechanism underlying the CoA-dependent transacylation system, we obtained the evidence that acyl-CoA is formed from membrane phospholipids through a CoA-dependent and ATP-independent process (*56, 67, 68*). When the microsomal fraction was incubated with CoA in the presence of bovine serum albumin, the accumulation of acyl-CoA was observed. The acyl moiety of the generated acyl-CoA was fatty acid esterified in microsomal phospholipids; lysophospholipids were concomitantly formed during the acyl-CoA formation (*67*). Acyl-CoA was formed in the absence of ATP and Mg^{2+}. Furthermore, free fatty acids failed to form acyl-CoA under the assay conditions used, suggesting that the formation of acyl-CoA from microsomal phospholipids and CoA is distinct from the synthesis by acyl-CoA synthetase. The enzyme responsible for the acyl-CoA synthesis may be regarded as a member of the phospholipase A since lysophospholipids were generated concomitantly. The fatty acyl moiety of acyl-CoAs formed during the incubation of liver microsomes with CoA resembles the fatty acid profile of the CoA-dependent transacylation system (20:4, 18:2, and 18:0). It is obvious that the CoA-dependent transacylation reaction consists of such acyl-CoA synthesis and subsequent reacylation of lysophospholipids with newly formed acyl-CoAs by acyl-CoA:lysophospholipid acyltransferases.

CoA-dependent, ATP-independent acyl-CoA synthesis is assumed to be mediated by the backward reaction of acyl-CoA:lysophospholipid acyltransferases. Several investigators, including us, have already pointed out the possible role of acyl-CoA:lysophospholipid acyltransferase operating in reverse in the formation of acyl-CoAs (*56, 67-69*), although there is no direct evidence. In order to make this point clearer, we attempted the solubilization of the CoA-dependent transacylation system from the microsomal fraction and the partial purification of the transacylation system. The CoA-dependent transacylation activity toward 1-acyl-GPI and acyl-CoA:1-acyl-GPI acyltransferase activity exhibited the same elution pattern at each chromatographic step (Yamashita *et al.*, manuscript in preparation). Furthermore, the CoA-dependent transacylation activity was augmented on the administration of clofibrate to rats (*70*), concomitant with an increase in acyl-CoA:1-acyl-GPC acyltransferase activity (*16, 49*). These results support the hypothesis that the CoA-dependent transacylation system consists of (i) acyl-CoA synthesis through the reverse action of acyl-CoA:lysophospholipid acyltransferase, and (ii) the transfer of the newly formed acyl-CoA to lysophospholipids through the forward action of acyl-CoA:lysophospholipid acyltransferase.

To date, the physiological meaning of the CoA-dependent transacylation system has not been fully elucidated. Further studies are needed to clarify this important issue. Previously, we suggested the possibility that CoA-dependent, ATP-independent acyl-CoA synthesis is involved in the modification of fatty acids once incorporated into phospholipids, such as desaturation and chain elongation (*68*). Sugiura *et al.* (*68*) proposed the occurrence of an alternative mechanism for the deacylation-reacylation cycle, consisting of CoA-dependent, ATP-independent acyl-CoA synthesis, and subsequent reacylation of lysophospholipids with the newly formed acyl-CoA, as shown in Fig. 2. Certain types of fatty acids of membrane phospholipids are continuously being transiently converted to an acyl-CoA without the consumption of ATP. The resultant acyl-CoA undergoes enzymatic modifications when the enzyme activities for desaturation and/or chain elongation are increased, leading to modification of the fatty acid composition of membrane phospholipids. This system may be effective in modifying fatty acids esterified in membrane phospholipids. On the other hand, free fatty acids released from membrane phospholipids or derived from other pools are converted to acyl-CoA with the consumption of ATP, and then undergo enzymatic modifications prior to incorporation into acceptor lipid molecules (Lands pathway, Fig. 2). These two pathways may act in concert in the modification of fatty acids esterified in membrane phospholipids in living cells. Whether or not this hypothesis is the case in various living tissues remains to be determined in the future.

3.3. Lysophospholipase/transacylase and phospholipase A2. Lysophospholipase catalyzes the hydrolysis of the carboxyl ester bonds of lysophospholipids such as lysophosphatidylcholine (LPC), producing fatty acids and water-soluble compounds such as GPC. This enzyme is involved in the dagradation of lysophospholipids which have toxic effects on biomembranes. This enzyme is widely distributed, and exists in a number of isoforms in various tissues and cells (*1, 6, 7*). Some isoforms of lysophospholipase are known to catalyze the transacylation between two LPC molecules leading to the formation of a diacylphospholipid and GPC, as shown in Fig. 1. Lysophospholipase/transacylase does not require any cofactors such as CoA. The transfer of a fatty acid from LPC to another may involve two step reactions (*71*). In the initial step, one molecule of LPC is cleaved into GPC and a fatty acid-enzyme covalent intermediate. The second step appears to involve the transfer of the fatty acid from the intermediate to LPC to produce PC. Alternately, the intermediate could be deacylated to produce a free fatty acid.

The cytosolic LPC:LPC transacylase purified from rat lung prefers palmitoyl-LPC to stearoyl-LPC, although LPC with acyl chains consisting of over 10 carbon atoms serve as acyl acceptors, while *sn*-glycero-3-phosphate and GPC do not (*72, 73*). The substrate, LPC, in the monomer form is hydrolyzed to a fatty acid, whereas the micellar form of LPC promotes the transacylation leading to the formation of PC. This enzyme may be, at least in part, involved in the production of the dipalmitoyl-GPC species of a pulmonary surfactant.

A lysophospholipase/transacylase has also been purified to near homogeneity from rabbit myocardium (*74*). The enzyme is important for the clearance of LPC. This enzyme was inhibited by a low concentration of acylcarnitine. The inhibition might be of pathological relevance since acylcarnitine is elevated in the ischemic myocardium; continuous accumulation of LPC during ischemia causes arrhythmia. A lysophospholipase/transacylase from rat liver cytosol is inhibited by phosphatidic acid in a competitive manner (*75*).

Phospholipase A2 cleaves fatty acids at the *sn*-2 position

of phospholipids to produce 1-radyl-2-lysophospholipid. However, there have been several reports concerning the acyltransferase and transacylase activities of phospholipase A2, and the involvement of phospholipase A2 in fatty acid remodeling of phospholipids. The human homologue of 30 kDa phospholipase A2 isolated from sheep platelets was cloned and homology analysis demonstrated that the cloned enzyme was a member of the 14-3-3 protein family (*76*). The recombinant protein catalyzed the cleavage of the *sn*-2 arachidonic acid of phospholipids through the formation of a stable acyl-enzyme intermediate. The isolation of the arachidonic acid-enzyme intermediate demonstrates the potential of the thioesterified arachidonic acid to be specifically transferred to putative nucleophilic acceptors including water (phospholipase A2 activity) and lipids (transacylation activity). Several extracellular phospholipase A2s including pancreatic phospholipase A2 were also shown to exhibit acyltransferase activity, from fatty acid to LPC, through the reverse reaction of phospholipase A2 in low polarity solvents (*77*). The results suggested the possible contribution of phospholipase A2 to the acyltransferase and transacylase activities in membranes, which resemble non-aqueous media. Furthermore, cytosolic and arachidonic acid-containing phospholipid-specific 85 kDa phospholipase A2 (cPLA2) is know to exhibit not only phospholipase A2 activity but also lysophospholipase/transacylase activity (*78*). Balsinde *et al.* (*79*) demonstrated that a specific inhibitor of calcium-independent phospholipase A2 prevents arachidonic acid incorporation and phospholipid remodeling in P388D1 macrophages, suggesting that calcium-independent phospholipase A2 is important for the remodeling of arachidonic acid-containing phospholipids.

3.4. CoA-independent transacylation reaction. Some tissues such as heart and brain, and inflammatory cells such as macrophages, platelets, neutrophils, and lymphocytes contain high amounts of ether-linked phospholipids. Generally, high amounts of arachidonic acid are found at the *sn*-2 position of these ether-linked phospholipids (*4*). Despite the predominance of arachidonic acid in the ether-linked phospholipids in the cells and tissues, the activities of acyl-CoA:1-alkyl-GPC and 1-alkenyl-GPE acyltransferase are very low (*44-46*). These results suggested that another mechanism may exist for the reacylation of ether-containing lysophospholipids.

The CoA-independent transacylation system was first described by Kramer *et al.* for human platelets (*80, 81*). Similar enzyme activity was also found in microsomes of several mammalian tissues and cells including macrophages (*57, 58, 82, 83*), neutrophils (*84, 85*), platelets (*86*), brain (*62, 63*), heart (*87*), testis (*88*), and Ehrlich cells (*89*). This system catalyzes the transfer of fatty acids from diacyl phospholipids to various lysophospholipids in the absence of any cofactors, differing from the CoA-dependent transacylation reaction which requires the presence of a CoA. The acyl donor is a fatty acid esterified at *sn*-2 position of diacylphospholipids but not a free fatty acid or an acyl-CoA in the CoA-independent transacylation system. The types of fatty acids transferred by the system are restricted to C20 and C22 polyunsaturated ones (*57, 58, 63, 81-83*). Both *n*-6 and *n*-3 acids can be transferred. Concerning the types of donor phospholipids, CGP, in particular diacyl-GPC, was shown to be the most preferred substrate (*57, 80*). Although diacyl-GPE also serves as a donor phospholipid to some extent, diacyl-GPI does not act at least when 1-alkyl-GPC is used as an acceptor. As for the acceptor lysophospholipids, three subclasses of CGP (1-alkenyl-GPC, 1-alkyl-GPC, and 1-acyl-GPC) were shown to be effective acceptors, 1-alkyl-GPC being most rapidly acylated among them (*57*). 1-Alkenyl-GPE, 1-alkyl-GPE, and 1-acyl-GPE were also acylated, 20:4 being transferred from diacyl-GPC. On the other hand, 1-acyl-GPI and 1-acyl-GPS, as well as 1-acyl-GP, do not serve as effective acceptors (*57, 80*).

The CoA-independent transacylation system was shown to be present in the microsomal fractions of various mammalian tissues but not liver (*56*), in which ether-containing phospholipids are known to be almost completely absent (*4*); the tissue distributions of the CoA-independent transacylation system and ether-containing phospholipids appear to be thus closely related. The fatty acid specificity of the CoA-independent transacylation reaction observed *in vitro* resembles the acylation pattern of ether-containing phospholipids (*4, 8, 57*). The CoA-independent transacylation system is considered to play an important role in the reacylation of ether-containing phospholipids to provide polyunsaturated fatty acid-containing ether phospholipids.

Another possible physiological role of the CoA-independent transacylation system is the prompt disposal of ether-containing lysophospholipids generated within a cell, being non-toxic diradylphospholipids formed, because ether-linked lysophospholipids are resistant to lysophospholipase. This is different from in the case of acyl-containing lysophopholipids, which can be rapidly metabolized by lysophospholipase or acyl-CoA:lysophospholipid acyltransferase.

Little information is available concerning regulation of the CoA-independent transacylation system. The treatment of platelets with phorbol myristate acetate or diacylglycerol enhances CoA-independent transacylation activity (*90*). Furthermore, the CoA-independent transacylation activity was also reported to be increased in human neutrophils after treatment with tumor necrosis factor-α (*91*).

The molecular nature of the enzyme involved in the CoA-independent transacylation system has not been determined. Purification of the enzyme protein has not yet been successful because the activity is sensitive to various detergents (*57*). However, we suppose the enzyme involved in the CoA-independent transacylation reaction is a member of the phospholipase A2 family since the transacylation system involves the degradation of diacyl phospholipids as the initial step. The CoA-independent transacylation activity is present in microsomes, being almost negligible in the cytosolic fraction (*57*), suggesting that the transacylation system is different from cPLA2. Winkler *et al.* (*92*) also reported that the CoA-independent transacylation activity could be biochemically and pharmacologically distinguished from cPLA2 and 14 kDa phospholipase A2.

3.5. The incorporation and mobilization of arachidonic acid in lipid classes. Arachidonic acid is a precursor of various eicosanoids with a wide variety of functions (*10*). The incorporation and distribution of arachidonic acid in phospholipids exhibit distinctive features, which are regulated by the remodeling system.

Arachidonic acid exogenously added to various types of cells was incorporated predominantly into the phospholipid fraction. In rabbit alveolar macrophages, arachidonic acid

was incorporated into the diacyl-GPC subclass and PI during short term incubation of intact cells with radiolabeled arachidonic acid (*93, 94*). When the prelabeled macrophages were chased in unlabeled medium, the gradual transfer of esterified arachidonic acid from the diacyl-GPC subclass to alkylacyl-GPC and alkenylacyl-GPE was observed (*93, 95*). Similar mobilization of arachidonic acid was observed in various types of cells such as neutrophils (*84, 85*), platelets (*86, 96*), HL-60 cells (*97*), and endothelial cells (*98, 99*). The mobilization of linoleic acid (18:2) from the diacyl-GPC subclass to alkylacyl-GPC and alkenylacyl-GPE was not observed (*93*). The main route of incorporation of arachidonic acid into diacyl-GPC during short term incubation comprised by the sequential reactions of acyl-CoA synthetase and acyl-CoA:1-acyl-GPC acyltransferase (Lands pathway). ATP, CoA, and Mg^{2+} were necessary for the formation of acyl-CoA from free fatty acids by acyl-CoA synthetase. Therefore, fatty acid incorporation through Lands pathway involves an ATP-dependent process. The subsequent transfer of arachidonic acid from the diacyl-GPC to alkylacyl-GPC and alkenylacyl-GPE was accounted for by the involvement of CoA-independent reactions, because the fatty acid specificity and acceptor specificity resemble those of the CoA-independent transacylation system assayed in an *in vitro* cell-free system. Such gradual transfer may account for the accumulation of arachidonic acid in ether-phospholipids such as alkylacyl-GPC and alkenylacyl-GPE in inflammatory cells (*57, 93*).

Similar mobilization of docosahexaenoic acid (22:6*n*-3) from the diacyl-GPC subclass to alkylacyl-GPC and alkenylacyl-GPE was observed in alveolar macrophages (*83*). Thus, the CoA-independent transacylation system also plays an important role in the mobilization of docosahexaenoic acid to alkylacyl-GPC and alkenylacyl-GPE. Cell cultures with docosahexaenoic acid in the medium showed an increase in docosahexaenoic acid and a decrease in arachidonic acid in phospholipids, in particular, alkylacyl-GPC and alkenylacyl-GPE (*100*). These results suggested that arachidonic acid and docosahexaenoic acid compete for a remodeling system, in particular, the CoA-independent transacylation system.

4. Synthesis of platelet-activating factor (PAF)

PAF was first described as a factor released from IgE-stimulated basophils that can trigger the aggregation of rabbit platelets (*101*). Its chemical structure was elucidated to be 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine in 1979 (*102-104*). PAF is a biologically active glycerophospholipid which is now assumed to act as a chemical mediator of anaphylaxis, septic shock and various inflammatory reactions. PAF also possesses potent anti-hypertensive activity and causes increases in vascular permeability (*5, 7, 9, 10*). The action of PAF is mediated *via* a specific receptor protein. Recently, cDNA encoding the PAF receptor was isolated from guinea pig lung (*105*). The PAF receptor is a member of the seven-transmembrane and G-protein coupled receptor family. The signal transduction pathway downstream of the PAF receptor was examined using receptor-expressed cells (*106*).

PAF is known to be synthesized *via* two synthetic routes, the remodeling pathway (deacylation-acetylation) and the *de novo* pathway (*107*). In inflammatory cells, such as neutrophils, the remodeling pathway is dominant (*5, 7, 10, 107*). These cells contain a considerable amount of alkylacyl-GPC, which has arachidonic acid at its *sn*-2 position (*4*). 1-Alkyl-2-arachidonoyl-GPC, a PAF precursor, is cleaved by phospholipase A2 into 1-alkyl-GPC (lysoPAF) and arachidonic acid. The *sn*-2 position of lysoPAF is sequentially acetylated by acetyl-CoA:lysoPAF acetyltransferase to yield PAF (*108*). PAF was degraded by PAF acetylhydrolase into lysoPAF, and subsequently acylated through the CoA-independent transacylation reaction to yield alkylacyl-GPC (PAF cycle, Fig. 3). A portion of the released arachidonic acid may be metabolized to prostaglandin(s) and leukotriene(s). The accumulation of arachidonic acid in alkylacyl-GPC would be favorable for the simultaneous synthesis of PAF and various eicosanoids. Thus, not only phospholipase A2 but also the CoA-independent transacylation reaction may trigger the biosynthesis of PAF. Sugiura *et al.* (*109*) reported that the amount of PAF produced increased to a level comparable to that produced by opsonized zymosan-stimulated polymorphonuclear leukocytes when 1-alkenyl-GPE was added to the cells. The addition of alkenyl-GPE to the cells caused the hydrolysis of alkylacyl-GPC to lysoPAF, which was presumably mediated by the CoA-independent transacylation system, and thereby led to the formation of PAF (Fig. 3). Similar results were obtained by Uemura *et al.* (*110*) and Nieto *et al.* (*111*).

Acetyl-CoA:lysoPAF acetyltransferase may be a member of the family of acyl-CoA:lysophospholipid acyltransferases, since the reactions are similar, *i.e.*, the donors are the CoA esters of carbonic acids and the acceptors are lysophospholipids. However, acetyl-CoA:lysoPAF acetyltransferase is clearly distinct from other acyl-CoA:lysophospholipid acyltransferases (*107, 108*). The enzyme requires Ca^{2+} for its activity. The enzyme activity is stimulated by various stimulatory agents (*112, 113*). The activation may be due to phosphorylation of the enzyme protein (*114*). Despite that acetyl-CoA:lysoPAF acetyltransferase is a key enzyme in PAF synthesis, the nature of the enzyme protein has not been well elucidated. Further studies are needed to precisely determine the mechanism underlying PAF synthesis.

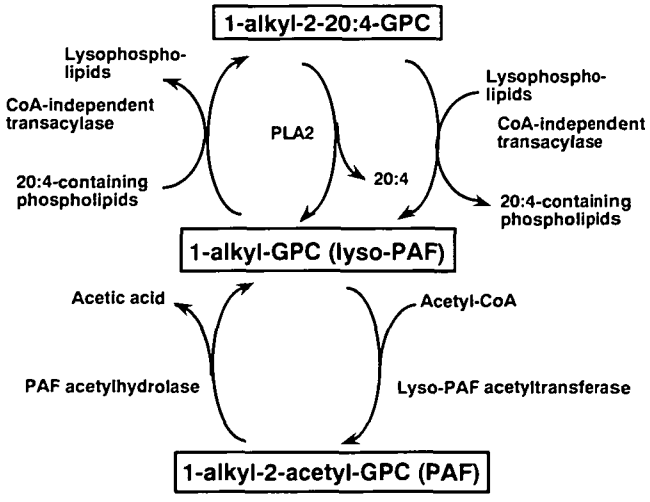

Fig. 3. **PAF cycle.**

Because the CoA-independent transacylation system is involved in the accumulation of arachidonic acid in 1-alkyl-2-arachidonoyl-GPC (*57, 93*), a common precursor of eicosanoids and PAF, and is possibly linked to the synthesis of PAF under some conditions (*109*), the enzyme may be a novel target for the development of anti-inflammatory drugs which could intervene in the production of various eicosanoids and PAF. Two structurally diverse inhibitors of CoA-independent transacylation activity, SK&F 98625 [diethyl 7-(3,4,5-triphenyl-2-oxo-2,3-dihydro-imidazole-1-yl)heptane phosphonate] and SK&F 45905 (2-[2-[3-(4-chloro-3-(trifluoromethyl)phenyl)-ureido]-4-(trifluoromethyl)phenoxy]-4,5-dichlorobenzene sulfonic acid), have been developed (*115*). Both SK&F 98625 and SK&F 45905 inhibit CoA-independent transacylation activity (IC50: 6-19 μM) competitively as to acceptor lysophospholipids, while the compounds have little or no effect on acyl-CoA: 1-acyl-GPC acyltransferase, acetyl-CoA:lyso PAF acetyltransferase, cyclooxygenase, or 14-kDa PLA2. In ionophore-stimulated neutrophils, SK&F 98625 and SK&F 45905 block the liberation of arachidonic acid from phospholipids, which suggests that the movement of arachidonic acid into specific phospholipid pools is a prerequisite for the release. SK&F 98625 and SK&F 45905 are able to decrease the prostaglandin production by several inflammatory cells. Both compounds also inhibit the production of PAF in ionophore-stimulated neutrophils and antigen-stimulated mast cells. These results show that the blockade of CoA-independent transacylation activity, which leads to inhibition of arachidonic acid-remodeling, results in inhibition of the production of lipid inflammatory mediators, *i.e.* eicosanoids and PAF.

The antiproliferative reagent, ET-18-O-CH_3 (1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine) causes similar inhibition of CoA-independent transacylation activity (IC50, 0.5 μM) (*116*). Including this compound, inhibitors of the CoA-independent transacylation system were found to inhibit cell proliferation and to induce apoptosis in HL-60 monocytic leukemia cells. These results suggest the possibility that the inhibition of the CoA-independent transacylation system and arachidonic acid-phospholipid remodeling may be linked to the blockade of proliferation and the induction of apoptosis in HL-60 cells. Whether or not such a possibility is the case should be determined in the future.

5. CoA-independent transacetylase

Lee *et al.* (*117*) identified a novel CoA-independent transacetylase in the membrane fraction of HL-60 cells that catalyzes the transfer of the acetate group of PAF to a variety of lysophospholipid acceptors. The CoA-independent transacetylase has no requirement for CoA, Ca^{2+}, or Mg^{2+}, and exhibits a broad pH optimum (7.0-8.0), with K_m values of 12.0 μM for PAF and 106.4 μM for lysoplasmalogens. Various lysophospholipids, such as 1-radyl-GPC, 1-radyl-GPE, 1-acyl-GPS, 1-acyl-GPI, and 1-radyl-GP, can also serve as acetate acceptors, showing relatively broad acceptor specificity compared with the CoA-independent transacylation system, which transfers long-chain acyl moieties. These differences in the substrate donor and acceptor specificities suggest that the CoA-independent transacetylase and the CoA-independent transacylation system represent two separate enzymes. The CoA-independent transacetylase may participate in the degradation of PAF and the fine tuning of PAF biological responses.

Furthermore, a similar CoA-independent transacetylase has been reported to enable the transfer of the acetate group of PAF to sphingosine, *N*-acetylsphingosine (C2-ceramide) being formed (*118*). C2-ceramide is produced *via* the transacetylase, and a physiological level of C2-ceramide is detected in intact HL-60 cells. Because C2-ceramide has many biological activities that differ from those of PAF and sphingosine, the CoA-independent, PAF-dependent transacetylase serves as a modifier of PAF and sphingosine functions by generating a variant lipid mediator, C2-ceramide.

6. *N*-Acylation reaction and biosynthesis of *N*-acylethanolamine

Schmid and co-workers reported that *N*-acylphosphatidylethanolamine (*N*-acyl PE) accumulate in infarcted canine heart (*119*). The *N*-acylation reaction is known to be involved in the biosynthesis of this type of lipid (*120-122*, Fig. 1D). The transacylase involved in the reaction catalyzes the direct transfer of the fatty acid from the *sn*-1 position of diacylphospholipids to the amino-group of PE. This transacylase does not require CoA but does need a millimolar concentration of Ca^{2+}. It is apparent that this transacylase is different from the CoA-independent transacylation reaction, which catalyzes the transfer of fatty acids from the *sn*-2 position of diacyl phospholipids to various lysophospholipids. The enzyme itself may be a phospholipase A1 since the transacylation reaction involves the degradation of diacyl phospholipids in initial step.

Schmid and co-workers also reported that saturated, monoenoic and dienoic species of *N*-acylethanolamine were formed through the phospholipase D-type reaction from the corresponding *N*-acyl PE (*119, 123*). *N*-Acylethanolamine accumulates in infarcted areas of canine hearts. In such areas, the mitochondria release abnormal amounts of Ca^{2+}. *N*-Acylethanolamine attenuates this release of Ca^{2+}. One possibility is that the accumulation of these lipids in damaged heart tissue may be a defence mechanism against cell damage induced by ischemia. *N*-Acylethanolamine also exhibits an antiinflammatory property.

Recently, Devane *et al.* (*124*) reported that the arachidonoyl species of *N*-acylethanolamine, "anandamide," is an endogenous cannabinoid receptor ligand. Anandamide exhibits various cannabimimetic activities *in vivo* and *in vitro*: for example, it inhibits forskolin-induced intracellular cyclic AMP accumulation, it inhibits *N*-type Ca^{2+} channels in several cells *in vitro*, and it induces antinociception, hypothermia, hypomotility, and catalepsy when administered to experimental animals.

The biosynthetic route for anandamide was proposed by several investigators (Fig. 4). First, in *in vitro* cell-free experiments, free arachidonic acid and ethanolamine were condensed to form anandamide (*125-128*). Another biosynthetic route for anandamide is the above mentioned *N*-acyl PE pathway (Schmid's pathway) involving Ca^{2+}-dependent transacylation and the phospholipase D-type reaction (*128-130*). We determined the contents and molecular species of *N*-acylethanolamine and *N*-acyl PE in rat brain and testis using a newly developed sensitive analytical method. Rat brain and testis contain not only saturated, monoenoic and dienoic species, but also ara-

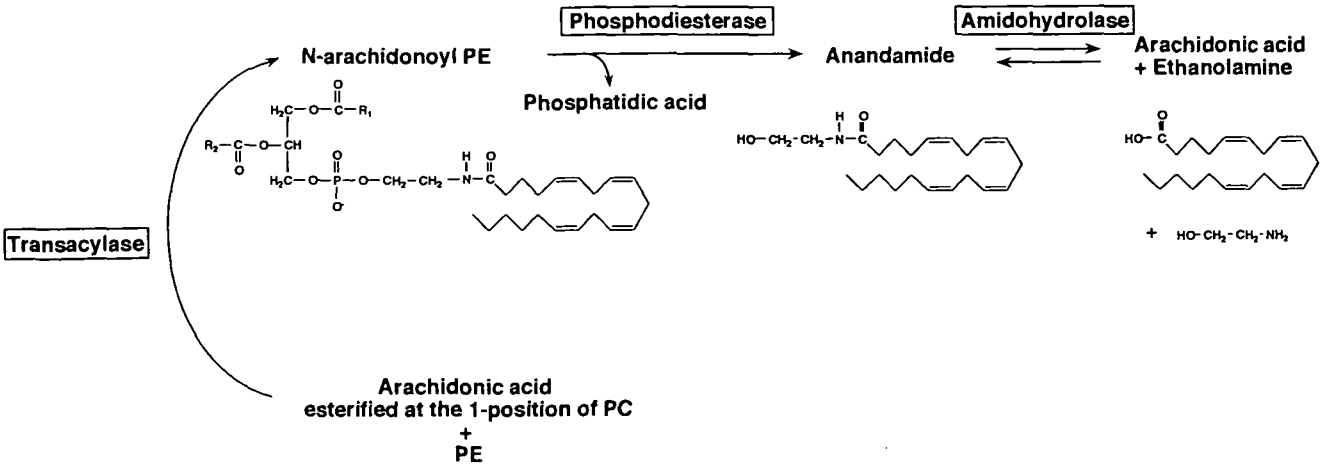

Fig. 4. **Biosynthesis and degradation of anandamide.**

chidonoyl species of *N*-acylethanolamine and *N*-acyl PE (*128, 130*). The fatty acid profile of *N*-acylethanolamine resembles those of *N*-acyl PE and fatty acids esterified at the *sn*-1 position of PC, but not those of free fatty acids in these tissues. The microsomal fractions of rat brain and testis contain enzyme activities of Ca^{2+}-dependent transacylase and the phospholipase D-type reaction. There is a possibility that the *N*-acyl PE pathway is the major synthetic route for anandamide in these tissues (Fig. 4).

7. Biological activities of long chain fatty acyl-CoAs and lysophospholipids

Long chain fatty acyl-CoAs and lysophospholipids, the substrates of acyl-CoA:lysophospholipid acyltransferases and the products of the reverse action of acyl-CoA:lysophospholipid acyltransferases, are known to exhibit a wide variety of biological activities. Long chain fatty acyl-CoAs are a metabolically active forms of fatty acids, and various kinds of enzymatic conversion of fatty acids, including desaturation, chain elongation and oxidation, proceed mainly in the form of fatty acyl-CoA in mammalian tissues (*131*). Another important role of acyl-CoAs is to serve as acyl donors in the formation of various types of simple and complex lipid molecules (*131*). In addition to their role as metabolic intermediates, long chain fatty acyl-CoAs are known to play several functional roles as modulators of various enzymes and cell functions. Possible roles of acyl-CoAs in the modulation of the activities of enzymes such as Na^+,K^+-ATPase (*132*), Ca^{2+}-ATPase (*133*), and protein kinase C (*134*), and the function of the thyroid hormone receptor (*135*) have been proposed. It has also been demonstrated that acyl-CoAs are required for the budding of transport vesicles from Golgi cisternae (*136*). Protein acylation such as myristoylation and palmitoylation involving acyl-CoAs was also reported to contribute significantly to the regulation of protein functions (*137*). Therefore, acyl-CoAs are a novel type of bioactive lipid having various functions and acting within the cell. The levels of acyl-CoAs are determined by acyl-CoA synthesizing and degradating enzymes. Not only acyl-CoA synthetase but also CoA-dependent, ATP-independent acyl-CoA synthesis are factors that increase the levels of acyl-CoAs, while acyl-CoA hydrolase and various acyl-CoA acyltransferases are factors that decrease the levels of acyl-CoAs. These enzyme activities may affect the cellular functions and enzyme activities described above.

Recently, we searched for novel targets of protein-acylation in rat liver microsomes and purified UDP-glucuronosyltransferase as a target of protein-acylation (*138, 139*). These proteins are acylated in the absence of other proteins, suggesting that they catalyze autoacylation, a novel type of protein acylation. The purified proteins also exhibit acyl-CoA binding activity. Treatment of the enzymes with acyl-CoAs suppresses the UDP-glucuronosyltransferase activity, suggesting that acyl-CoAs are modulators of UDP-glucuronosyltransferase. Further studies on the key compounds, acyl-CoAs, should lead to a precise understanding of the relevance/crosstalk of fatty acid metabolism and glucuronidation.

There have been several reports on the pharmacological effects of lysophospholipids (*10, 140-149*). LPC is cytolytic, therefore, the level of LPC within cells must be under strict control. LPC is synthesized through several routes, such as the cleavage of PC by phospholipase A2 or the CoA-dependent and CoA-independent transacylation system, and is cleared off by lysophospholipase and acyl-CoA:LPC acyltransferase. In the blood circulation of mammals, however, a substantial amount of LPC exists as a complex with albumin. The liver is the source of much of the LPC that enriches unsaturated fatty acids associated with albumin in the blood (*140*). Also, LPC is synthesized through the cleavage of PC by lecithin cholesterol acyltransferase (*10*). LPC induces a hypotensive response in rats (*141*), inhibits platelet aggregation (*10*), potentiates the mitogenic effect of diacylglycerol in T-lymphocytes (*142*), and enhances the differentiation of HL-60 cells into macrophages (*143*). LPC in oxidized low density lipoproteins exerts diverse effects on monocytes/macrophages and vascular endothelial cells, in relation to the promotion of atherosclerosis. The effects may be, at least in part, mediated by modulation by LPC of the expression of various genes, such as various adhesion molecules, heparin-binding epidermal growth factor-like growth factor, and platelet-derived growth factor (*144*). LPC has been also shown to accumulate in myocardial tissue during ischemia, and thus to become toxic to the heart, leading to arrhythmia and abnormalities of relaxation (*74*).

Conclusion

In this review, we summarized the properties of the acyltransferases and transacylases involved in the fatty acid remodeling of phospholipids, and the metabolism of bioactive lipids and their precursors. It is clear from the numerous studies described in this review that acyl-CoA: lysophospholipid acyltransferases, CoA-dependent and CoA-independent transacylation systems, and lysophospholipase/transacylase play important roles in the remodeling of membrane phospholipids. The molecular species composition of each phospholipid in various tissues and cells is thought to be determined by the levels of these enzyme systems, which exhibit different acyl donor and acyl acceptor selectivities. Several physiological roles of the fatty acid remodeling itself have been postulated; one of them is regulation of the physicochemical properties of membranes such as membrane fluidity, and another significant one is regulation of the distribution and accumulation of physiologically important fatty acids such as arachidonic acid. However, a thorough understanding of the physiological role of fatty acid remodeling has not been attained yet.

These acyltransferases and transacylases are involved in not only the remodeling of fatty acids but also the synthesis of some of bioactive lipids. For example, *N*-acylethanolamines, including anandamide, are synthesized *via* a transacylase-mediated pathway. The levels of lysophospholipids also seem to be regulated by acyltransferases and transacylases. Acyl-CoAs, unique bioactive lipids may also be synthesized through the CoA-dependent transacylation system.

So far, available information on the properties of these remodeling systems, including substrate specificities, and of the biosynthetic routes for these bioactive lipids have been obtained through either whole cell studies or the use of subcellular fractions. Research on the molecular mechanisms of these enzymes responsible for the remodeling and biosynthesis of these bioactive lipids has been limited. Complementary DNA cloning of these enzymes was not fully successful because the purification of these enzymes was difficult. Clearly the next step in our study is the elucidation of the molecular natures of these enzymes, including purification and cDNA cloning, which will lead to a more precise understanding of their physiological roles in fatty acid remodeling and the synthesis of bioactive lipids.

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