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THE ROLE OF TRANSACYLASES IN THE METABOLISM OF ARACHIDONATE AND PLATELET ACTIVATING FACTOR

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I. INTRODUCTION

The incorporation of fatty acids into phospholipids can occur via acyl-CoA acyltransferases and by CoA-dependent and CoA-independent transacylases. These three different types of acylation reactions are illustrated in Fig. 1. The enzyme that utilizes acyl-CoAs as a substrate is called an acyl-CoA acyltransferase; the terms transacylase and acyltransferase should never be used as synonyms since they refer to two distinctly different acylation mechanisms. This review centers on the CoA-dependent and CoA-independent transacylases; the acyl-CoA:lyso-phospholipid acyltransferases are discussed primarily for the lyso-phospholipid acyl acceptors that contain ether bonds since the extensive studies with acyllyso-phospholipids as substrates for acyl-CoA acyltransferase are beyond the scope of this article.

Transacylases catalyze the transfer of polyunsaturated fatty acids from one phospholipid to another without the release of any free fatty acids. The lyso-phospholipid that serves as the acyl acceptor molecule for the transferring acyl group is thought to be generated by a putative phospholipase A_2, but the precise mechanisms responsible for these reaction steps are presently unknown. Most recently a transacylation step has been directly linked to the formation of lyso-PAF and the subsequent production of PAF. These studies have led to the formulation of a novel sequence of enzymatic reactions (see Section VII) that involve both phospholipase A_2 and transacylase activities.[141]

In order to better understand the involvement of transacylases in the metabolism of arachidonic acid and PAF, we have also provided background on the polyunsaturated fatty acid composition of phospholipids and on factors that influence the movement of arachidonate through various glycerolipids. Although the role of transacylases in the cellular trafficking of arachidonate between different phospholipids is still poorly understood, it is becoming increasingly apparent that transacylation reactions are of primary importance. Our intent is to provide a concise overview of what is currently known about

1) Acyl-CoA Acyltransferase

2) CoA-dependent Transacylase

3) CoA-independent Transacylases

FIG. 1. The three different mechanisms responsible for the acylation of lyso-phospholipids: (1) acyl-CoA acyltransferase, (2) CoA-dependent transacylase, and (3) CoA-independent transacylase (a) acyl transfer between two lyso-phospholipid molecules and (b) acyl transfer between an intact phospholipid and a lyso-phospholipid.

the CoA-independent and CoA-dependent transacylases in mammalian systems. Additional information regarding transacylation, deacylation-reacylation, and phospholipid remodeling can be found in a recent review by McDonald and Sprecher.[79a]

II. POLYUNSATURATES IN VARIOUS LIPID CLASSES AND SUBCLASSES

Diacylglycerophosphatides from mammalian tissues are generally more enriched in polyunsaturated acyl groups (>2 double bonds per acyl chain) than are triacylglycerols from the same tissue.[52] These polyunsaturated fatty acids of phosphatides are primarily esterified to the *sn*-2 position of the glycerol moiety. In addition to the usually more abundant diacyl subclasses, cells from most tissues also contain ether-linked phospholipid subclasses. The presence of ether-linked lipids in cellular lipids was largely a curiosity before the discovery of PAF in 1979, an event that has stimulated a great interest in glycerolipids containing ether bonds. Diradyl-GPC and diradyl-GPE are the two glycerophospholipid classes that have been analyzed extensively for the acyl composition of their ether-linked subclasses, i.e. alkylacyl- (plasmanyl) and alk-1-enylacyl- (plasmenyl) groups. Acyl compositions of subclasses of diradyl-GPC and diradyl-GPE from a variety

TABLE 1. Percent of Arachidonate in Subclasses of Diradyl-GPC and Diradyl-GPE

Tissue	Diacyl-GPC*	Alkylacyl-GPC	Diacyl-GPE*	Alk-1-enylacyl-GPE
Macrophages (Nakagawa *et al.*, 1985b)[88]	11.2	51.5	19.9	63.1
P388D$_1$ Cells (Blank *et al.*, 1989)[13]	0.6	1.7	7.2	27.9
HL-60 Cells (Suga *et al.*, 1990)[128]	16.4	20.3	14.8	28.2
Platelets (Mueller *et al.*, 1983)[85]	23.2	43.7	60.0	68.3
Neutrophils (Mueller *et al.*, 1984)[84]	4.4	10.4	13.2	32.7
Ehrlich Ascites Cells (Wood and Snyder, 1969)[157]	9.3	22.6	9.3	25.1
FMA3 Mastocytoma Cells (Yoshioka *et al.*, 1985)[161]	9.1	18.3	5.3	26.6
P-815 Mastocytoma Cells (Yoshioka *et al.*, 1985)[161]	6.8	31.4	12.3	40.0

*Assumes all arachidonate in the diacyl subclasses is located in the *sn*-2 position of glycerol; percentages of arachidonate that were based upon *sn*-1 plus *sn*-2 positions were multiplied by a factor of two so they would be directly comparable to the etheracyl subclasses.

of tissues have been previously reviewed.[53,54,135] In fact, there is little new information in the literature that would contribute significantly to the overall patterns of acyl composition of choline and ethanolamine phosphatides described in these excellent reviews. A few selected examples for different cells showing the percentage of arachidonate in the *sn*-2 position acyl groups of subclasses of diradyl-GPC and -GPE are shown in Table 1. Generalizations that can be made from the extensive data in these reviews include: (1) the ether-linked subclasses of diradyl-GPE and -GPC usually contain increased percentages of polyunsaturated fatty acids when compared to their diacyl subclasses; (2) subclasses of diradyl-GPE often contain greater percentages of polyunsaturated fatty acids than the corresponding subclasses from diradyl-GPC; and (3) the ratio of 16:0 to 18:0 alkyl and/or alk-1-enyl chains is higher in the -GPC subclasses than in the -GPE subclasses from the same tissue. As with most generalizations, there are exceptions; for example, it was found in rat myocytes the plasmenyl species of 1-radyl-2-acyl-GPC and 1-radyl-2-acyl-GPE were both highly unsaturated at the *sn*-2 position.[68]

A major emphasis in much of the earlier, as well as current, research efforts has been directed toward understanding how arachidonic acid is incorporated, distributed, and metabolized in the different lipid classes and subclasses. This emphasis is understandable considering the biological potency of its cyclo-oxygenase/lipoxygenase metabolites and the close association of arachidonic acid metabolism with the biosynthesis of platelet activating factor (see Section VII). However, it should be remembered that, even though this review mainly concentrates on arachidonic acid, other polyunsaturates such as eicosapentaenoic and docosahexaenoic acids are also enriched in the ether-linked phospholipids. In fact, current evidence indicates eicosapentaenoate and arachidonate are metabolized in a similar manner in rat neutrophils[20,21] and that they are both transferred from diacyl-GPC to alk-1-enylacyl-GPE in human platelets following thrombin stimulation.[153] Docosahexaenoic acid is particularly enriched in the plasmenylethanolamine subclass from brain[53,78,94] and can be increased by supplementation of the 22:6 acid to represent nearly one-half of the plasmenylethanolamine subclass of P388D_1 cells[13] or rat hearts.[160] We have also found that PAF biosynthesis in arachidonate-depleted HL-60 cells (differentiated granulocytic form) could be induced (3- to 5-fold) not only by arachidonic acid repletion but also by supplementation with other polyunsaturated fatty acids.[128] Therefore, it appears likely that information on the incorporation, cellular distribution, interlipid-trafficking, and release of arachidonic acid can also be applied to other polyunsaturated fatty acids.

Another group of unique polyunsaturated phospholipid molecules are those containing polyenoic acyl groups at both the *sn*-1 and *sn*-2 positions of glycerol. Aveldano and Bazan[7] reported that almost 30% of the phosphoglycerides in rod outer segments from bovine retina were dipolyunsaturated. Similar results were obtained from rod outer segments of the frog retina where at least 50% of the phosphatidylethanolamine consisted of dipolyunsaturated molecular species.[155] Docosahexaenoic acid appears to be the major component of dipolyunsaturated species in retinal phospholipids. Didocosahexaenoyl species of phospholipids also occur in rat retinal membranes.[127] However, dipolyunsaturated molecular species of glycerophosphatides are not only limited to retinal tissues since diarachidonoyl species of phospholipids have also been found in rat testes,[12] rat erythrocytes,[116] and human neutrophils.[29] The biological role of these novel, highly unsaturated phospholipids remains to be elucidated.

III. THE MOVEMENT OF ARACHIDONIC ACID AMONG LIPID CLASSES IN INTACT CELLS

Incorporation of arachidonic acid into cellular glycerolipids requires that it first be converted to the CoA derivative (i.e. arachidonoyl-CoA), the substrate for acyltransferases. After arachidonate is incorporated into glycerolipids, the arachidonate moiety is usually transferred from one lipid class to another by processes involving acyl group replacement generally referred to as the "remodeling" pathway. This movement of

arachidonic acid between lipid classes involves acyl-CoA acyltransferases, CoA-dependent transacylases, and CoA-independent transacylases as pointed out in Section 1. This section reviews the interlipid movements of arachidonic acid that have been described in intact cells and, therefore, specific acylation mechanisms involved are not apparent. However, enzymatic studies with some of the same cells, as described in Sections V and VI, will shed some light on this issue. Irvine[58] has aptly pointed out the difficulties encountered in interpreting experimental results using radiolabeled arachidonic acid and, since radioactive (^{3}H or ^{14}C) arachidonic acid has been used almost exclusively to monitor the movement or trafficking of arachidonate among various cellular lipids, these caveats should be borne in mind by the reader. Our laboratory has also observed that several factors influence the lipid distribution and interlipid movement of radiolabeled arachidonic acid in HL-60 cells depleted of arachidonate by continual growth in serum-free media.[14] One important consideration is the concentration of arachidonic acid used since very low concentrations ($<0.1\ \mu M$) tended to equilibrate more quickly and, unlike higher concentrations ($10\ \mu M$), produced no labeled triacylglycerols. Moreover, increasing endogenous cellular levels of unlabeled arachidonate in the various lipid classes through media supplements affected subsequent experimental results obtained with low concentrations (10 nM) of radiolabeled arachidonic acid so that they resembled data from short-term experiments performed with higher concentrations of [^{3}H]arachidonic acid. Increasing the concentration of suspended cells gave results analogous to those obtained when the concentration of [^{3}H]arachidonic acid was decreased in the media. Also, when the HL-60 cells were differentiated into a granulocytic form with dimethylsulfoxide, less [^{3}H]arachidonate was found in the total phospholipids, but the proportion of radioactivity associated with the ether-linked phospholipids (alkylacylglycerophosphocholines and plasmalogens) was much higher than in the undifferentiated cells.

Exogenous arachidonic acid is usually rapidly and predominately incorporated mainly into cellular glycerophospholipids. However, there are numerous reports that describe the incorporation of radiolabeled arachidonic acid into triacylglycerols as well as phospholipids. The amount of arachidonate incorporated into triacylglycerols by human neutrophils[29] and bovine endothelial cells[40] had a positive correlation with the media concentration of arachidonic acid. Arachidonate present in triacylglycerols is subsequently transferred to cellular phospholipids in human neutrophils,[72] neuroblastoma cells,[34,125] endothelial cells,[40,124] and alveolar macrophages.[8] Increased incorporation of arachidonic acid into cellular triacylglycerols with higher media concentrations of arachidonic acid together with the eventual transfer of the arachidonate from triacylglycerols to phospholipids is consistent with triacylglycerols acting as a storage site when higher than physiological levels of arachidonic acid are present. Cook and Spence[35] show that both the ^{3}H of glycerol and ^{14}C of linoleate in double labeled triacylglycerols are transferred to phospholipids by neuroblastoma cells. These results suggest that a direct transfer of linoleate from triacylglycerols to phospholipids via a transacylase mechanism is not likely, but instead a triacylglycerol lipase might produce diacylglycerols that can then be utilized by either choline- or ethanolamine-phosphotransferases via *de novo* synthesis of the appropriate phospholipid. Whether a similar transfer of both glycerol and arachidonate moieties of triacylglycerols to phospholipids occurs is presently unknown.

In contrast to the movement of arachidonate from triacylglycerols to phospholipids, guinea pig alveolar macrophages labeled by incubation with vesicles (liposomes) of 1-acyl-2-[1-^{14}C]arachidonoyl-GPC, transferred the [^{14}C]arachidonic acid from the diacyl-GPC to both diradyl-GPE (22%) and triacylglycerols (12%).[92] The presence of free arachidonic acid inhibited the transfer of [^{14}C]arachidonic acid to triacylglycerols but had no effect on the transfer of [^{14}C]arachidonate from diacyl-GPC into diradyl-GPE; these results suggest two different mechanisms are involved in the arachidonate transfer, i.e. acyl-CoA acyltransferase in transfer to triacylglycerols vs. a transacylase for transfer between phospholipids. Sivarajan *et al.*[124] observed a transfer of arachidonate from phospholipids to triacylglycerols in bovine endothelial cells that were prelabeled with [1-^{14}C]arachidonic acid and then treated with the ionophore A23187. They

suggested triacylglycerols might act as a storage site for arachidonate during times of cellular stress.

Diradyl-GPC is the most extensively labeled phospholipid class during short term (0.5–2 hr) incubations of most cells with radiolabeled arachidonic acid.[3,11,18,28,30,31,33,36,44,46,62,63,66,72,86,87,104,113,114,123,130,137,140,154] In experiments where the distribution of radiolabeled arachidonate in the subclasses of diradyl-GPC and diradyl-GPE has been determined, it was found the diacyl-GPC subclass, rather than the alk-1-enylacyl-GPC or alkylacyl-GPC subclasses, was labeled to the greatest extent, particularly at early times. When incubations of arachidonate prelabeled cells were continued in unlabeled media, there was a movement of esterified arachidonate as indicated by a loss of radioactivity from the diacyl-GPC subclass and a gain of radioactivity in the alk-1-enylacyl-GPE and/or alkylacyl-GPC subclasses in human neutrophils,[27,28,72] rat platelets,[31,33,36] rat lymphocytes,[44] rabbit alveolar macrophages,[87,130] human leukemic HL-60 cells,[128] endothelial cells,[137,154] and murine mastocytoma P-815 cells.[162] This movement of arachidonic acid from diacyl-GPC into the ether-linked subclasses of diradyl-GPC and diradyl-GPE could explain the higher endogenous concentrations of arachidonate that are usually associated with the ether-linked phospholipids.

There must be some utilization or turnover of the arachidonic acid that is in the ether-linked phospholipids or the movement of arachidonate from diacyl to etheracyl phospholipids would soon reach its capacity. In this regard, there are reports suggesting that in some cells alk-1-enylacyl-GPE participates in the agonist-stimulated release of arachidonic acid for the subsequent production of eicosanoids.[17,18,30,37,62,63,113,114,138] However, at this time, there is no consensus that the ethanolamine plasmalogens are a predominate donor of arachidonic acid for the lipoxygenase and cyclo-oxygenase pathways in most tissues. It has also been established that another ether-linked phospholipid, alkylarachidonoyl-GPC, serves as the primary precursor of PAF in the remodeling pathway of biosynthesis of PAF by inflammatory cells[3,26,87,104,136] and differentiated HL-60 cells.[128] Therefore, the synthesis of PAF via the remodeling pathway would also provide a mechanism for the transfer of arachidonic acid to other phospholipids from the ether-linked alkylacyl-GPC pool. The agonist stimulated release of arachidonic acid from ethanolamine and/or choline phospholipids is likely initiated by a phospholipase A_2 activity. This phospholipase A_2 activity may resemble the recently described plasmalogen-selective phospholipase A_2 found in ischemic rabbit heart.[51] Moreover, an arachidonoyl-specific phospholipase A_2 activity has been purified from leukemic U937 cells[42] and from RAW 264.7 cells,[70] but neither show a preference for alkylacyl vs. diacyl phospholipids.

Agonist stimulated release of arachidonic acid is often accompanied by an apparent movement of arachidonate caused by the rapid reacylation of the released arachidonate back into cellular lipids.[58] This redistribution of labeled arachidonate makes it very difficult to determine which lipids are the actual targets for the initial agonist stimulated arachidonate release unless both mass and radioactivity measurements are made (e.g. specific radioactivities). Examples of at least moderately successful attempts to block the reacylation of released arachidonic acid by intact cells have been made using an albumin "trap",[60] *p*-hydroxymercuribenzoate,[55,66] ethylmercurisalicylate,[47] or *N*-ethylmaleimide.[47] Because these compounds, particularly the latter three, are likely to influence other cellular metabolic events too, caution must be observed in interpretation of experimental results obtained with these inhibitors. *N*-ethylmaleimide is believed to inhibit the CoA-independent transacylase activity present in membranes isolated from human platelets[63,64] and the Fischer R-3259 sarcoma.[69]

Although much progress has been made during the last ten years in defining the movement of arachidonic acid among different cellular lipid classes and subclasses, the results have required reappraisal of some older dogmas and development of novel pathways to fit the experimental data. Recently developed methodology allowing accurate measurement of not only phospholipid subclasses but also molecular species within the subclasses of phospholipids should provide important new information about the trafficking of polyunsaturated fatty acids among cellular lipids.

IV. ACYLATION OF LYSO-PAF AND RELATED LYSO-PHOSPHOLIPIDS VIA ACYL-CoA ACYLTRANSFERASE

Lands first reported an acyltransferase that could acylate acyllyso-GPC with acyl-CoA as the cosubstrate in rat liver microsomes.[67] However, the direct evidence for the acylation of alkyllyso-GPC by an acyl-CoA dependent acyltransferase was demonstrated by Waku and Nakagawa[150] in rabbit sarcoplasmic reticulum. Thereafter, acyl-CoA:alkyllyso-GPC acyltransferase activity was shown to be present in the microsomal fraction of Ehrlich ascites tumor cells,[151,152] microsomal preparations and mitochondrial supernatant fractions from rat testes,[159] membrane fractions of human platelets,[80] rat platelet homogenates,[36] rat brain microsomes,[94] the membrane fractions of human neutrophils,[27] rabbit alveolar macrophages,[115] and Fischer sarcoma microsomes.[69]

Acyl-CoA:alkyllyso-GPC acyltransferase has been well-characterized in human platelets.[80] This enzyme is localized in the high speed microsomal fraction (150,000 × g, 1 hr) with a pH optimum of 7.5. It is inhibited by Ca^{2+}, with a 60% decrease from controls at 2 mM and is slightly stimulated by 1 mM EDTA (112% of the control). Maximal formation of alkylacyl-GPC is observed at 150 μM alkyllyso-GPC and 20 μM unsaturated acyl-CoA. Most other studies on acyl-CoA:alkyllyso-GPC acyltransferase center on the substrate specificities of acyl-CoAs and comparison of the acylation rates with acyllyso-GPC or alk-1-enyllyso-GPC as substrates. Acyl-CoA:alkyllyso-GPC acyltransferase shows a high and similar acylation rate for polyunsaturated acyl-CoAs such as 18:2-CoA, 18:3-CoA, and 20:4-CoA, but little activity for saturated acyl-CoAs and 18:1-CoA.[80,150,152,159] Yet, when hexadecyllyso-GPC is acylated by an acyl-CoA acyltransferase utilizing the endogenous fatty acid pool, significant amounts of hexadecylacyl-GPC with molecular species of 16:0–16:0, and 16:0–18:1 are formed in the presence of CoA + ATP + $MgCl_2$.[69,94,115] Obviously, further studies are required to resolve the discrepancy between these two observations.

Acyl-CoA:alkyllyso-GPC acyltransferase activity is considerably lower when compared with those acyltransferases that acylate acyllyso-GPC,[27,36,80,150] and alk-1-enyllyso-GPC.[150] The specific activity of linoleoyl-CoA:alkyllyso-GPC acyltransferase in rabbit sarcoplasmic reticulum represents only 2% of the linoleoyl-CoA:acyllyso-GPC acyltransferase and 4% of linoleoyl-CoA:alk-1-enyllyso-GPC acyltransferase.[150] The rate of fatty acyl groups transferred to lyso-PAF is 3–14 times slower than to acyllyso-GPC in membrane fractions prepared from human platelets.[80,81]

It is not clear at present whether a single enzyme catalyzes the transfer of the acyl group from acyl-CoA to various lyso-phospholipids or if a unique enzyme activity is responsible for selectively acylating alkyllyso-GPC. However, based on pH profiles, acyl-CoA specificities, heat inactivation studies as well as the differential effect of modulation of *N*-ethylmaleimide, dithiothreitol, and GSH[6] it is postulated that alk-1-enyllyso-GPE acyltransferase and acyllyso-GPE acyltransferase in guinea pig heart microsomes represent different enzymes. Similar conclusions have been reached for alk-1-enyllyso-GPE acyltransferase and alk-1-enyllyso-GPC acyltransferase due to their different pH optima and heat inactivation patterns.[5,6] Also, even though acyllyso-GPC acyltransferase and acyllyso-GPE acyltransferase have similar pH profiles and susceptibilities to heat, the acyllyso-GPE acyltransferase activity is inhibited in a noncompetitive manner by acyllyso-GPC; thus, it appears that acylations of acyllyso-GPC and acyllyso-GPE are catalyzed by different enzymes.[6] Finally, alk-1-enyllyso-GPC acyltransferase and acyllyso-GPC acyltransferase do not appear to be identical catalytic proteins since the two enzyme activities exhibit different responses to cations, detergents, and heat treatments.[6] Therefore, the possibility exists that a novel enzyme catalyzes the acylation of alkyllyso-GPC by acyl-CoA.

Similarly, results from studies of acyllyso-GPC acyltransferase suggest that there are several acyltransferases specific to individual acyl-CoAs. For example, enzyme stability[112] and the effect of organic solvents[61] on the rat liver microsomal acyltransferase activity vary with the species of acyl-CoA used as the acyl donor. Also, oleoyl-CoA:acyllyso-GPC acyltransferase is much more susceptible to Triton X-100 inhibition than are linoleoyl-CoA

or arachidonoyl-CoA acyltransferase.[96] In bovine brain, highly purified acyllyso-GPC acyltransferase from solubilized microsomes shows high selectivity for 20:4-CoA and does not use either 18:0-CoA or 16:0-CoA as substrates.[39] Furthermore, oleoyl-CoA:acyllyso-GPC acyltransferase can be activated 2- to 3-fold by treatment of human neutrophils with ionophore A23187 and modulated by phosphorylation and dephosphorylation; the transfer of oleoyl-CoA to acyllyso-GPC is not inhibited by arachidonoyl-CoA and arachidonoyl-CoA:acyllyso-GPC acyltransferase is not altered by ionophore A23187-stimulation of PMN.[111] These data suggest separate acyl-CoA acyltransferase activities catalyze the transfer of oleoyl- and arachidonoyl-CoAs to acyllyso-GPC in human neutrophils. Additional investigation is needed to determine if several acyltransferases exist and whether each is selective for individual acyl-CoAs in the acylation of alkyllyso-GPC.

V. CoA-DEPENDENT TRANSACYLATION REACTIONS

Irvine and Dawson[59] were the first to describe a direct CoA-dependent transfer of an *sn*-2 acyl moiety of an intact phospholipid to a lyso-phospholipid (Fig. 2). The lyso-phospholipid acceptor in these initial experiments with rat liver microsomes as the enzyme source was either lyso-phosphatidylcholine or lyso-phosphatidylinositol. The acyl transfer was clearly due to a true transacylation reaction since free fatty acids or a phospholipase A_2 activity were shown not involved. The transfer rate of arachidonate from phosphatidylinositol to lyso-phosphatidylcholine or from phosphatidylcholine to lyso-phosphatidylinositol was ca. four times greater than the transfer rate for linoleate between the same pairs of phospholipids. Transfer of oleate to the lyso-phospholipid acceptor molecules under identical experimental conditions did not occur. Irvine and Dawson[59] envisioned the mechanism for the CoA-dependent acylation of lyso-phospholipids in these experiments to be the reversal of steps in the well-established acyl-CoA acyltransferase reaction as illustrated for arachidonate in Fig. 2; this sequence involves the initial transfer of arachidonate from an intact phospholipid to CoA (Fig. 2, Rx I), followed by the transfer of the arachidonate from the acyl-CoA to an appropriate lyso-phospholipid acceptor (Fig. 2, Rx II).

An active CoA-dependent transacylase was described in mouse lymphocytes that could catalyze the transfer of arachidonate from phosphatidylcholine to lyso-phosphatidylethanolamine.[140] Similar types of experiments with membranes prelabeled with [^{14}C]oleic acid showed no transfer of the oleate from phosphatidylcholine to lyso-phosphatidylethanolamine. However, unlike the results reported for rat liver microsomes and endogenous donor phospholipids,[59] there was no substantial transfer of labeled arachidonate from phosphatidylinositol to either lyso-phosphatidylcholine or lyso-phosphatidylethanolamine by the lymphocyte membranes;[140] also, no significant transfer of arachidonate occurred with phosphatidylcholine or phosphatidylethanolamine as the donors and lyso-phosphatidylinositol as the acceptor.

Several papers have characterized some of the properties of CoA-mediated transacylation reactions in human[65] and rat[16,32,76] platelets. Kramer and coworkers[65] found that membranes from human platelets exhibited a preference for lyso-phosphatidylserine as the arachidonate acceptor in the CoA-dependent transacylase reaction; however, other

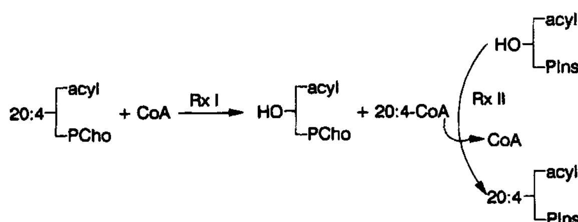

FIG. 2. Example of a reaction catalyzed by an intact phospholipid:lyso-phospholipid CoA-dependent transacylase.

lysophospholipids were also utilized as acyl acceptors by the CoA-dependent transacylase, albeit at lower rates in the order of lyso-phosphatidylethanolamine > lyso-plasmenylethanolamine > lyso-phosphatidylinositol. The K_m for lyso-phosphatidylserine and CoA in this transacylation reaction were 76 and 1.4 μM, respectively. In contrast, the CoA-independent catalyzed transacylation reaction in human platelets had an extremely high preference for lyso-plasmalogen as the arachidonate acceptor.[63,64]

In rat platelet homogenates, the CoA-dependent transacylation of [^{14}C]arachidonate between intact phospholipids and lyso-phospholipids exhibited a more efficient rate of transfer of arachidonate from phosphatidylcholine to the ethanolamine-containing phospholipids than from phosphatidylinositol.[32] Lyso-phosphatidylserine also served as a good acceptor molecule for arachidonate under these conditions with a concomitant decrease in the radiolabeled arachidonate associated with phosphatidylcholine. Addition of lyso-phosphatidylcholine to platelet homogenates caused a decrease in the amount of label found in phosphatidylinositol and to a lesser degree in the ethanolamine phospholipids; a corresponding increase was observed for the labeled arachidonate associated with phosphatidylcholine. In contrast, lyso-phosphatidylinositol and lyso-phosphatidic acid did not serve as acyl acceptors in this sytem. Noteworthy is that free unlabeled arachidonic acid did not influence the transfer of [^{14}C]arachidonate between the two phospholipid substrates utilized by the CoA-dependent transacylases.

Other studies with rat platelet sonicates demonstrated that a decrease in the CoA-dependent transacylation of palmitoyllysoglycerophosphocholine as a function of Ca^{2+} concentration was due to an increased level of endogenous lyso-phospholipids presumably generated by a Ca^{2+}-dependent phospholipase A_2.[16] Results from these experiments also revealed that CoA induced the transfer of [^{14}C]aracidonate from phosphatidylcholine to alkyllysoglycerophosphoethanolamine when the latter was added exogenously or formed endogenously after adding Ca^{2+} to the incubation mixtures. Based on these findings, the authors[16] suggested the CoA-dependent transacylation reaction involving phosphatidylcholine as the donor of arachidonate involved two steps: (a) formation of the lyso-phospholipid acyl acceptor molecule via a Ca^{2+} dependent phospholipase A_2 and (b) the initiation of the arachidonate transfer from phosphatidylcholine to lyso-phosphatidylethanolamine for the formation of phosphatidylethanolamine.

Molecular species analysis[76] of the phosphatidylcholines formed via the CoA-dependent transacylase in rat platelet homogenates revealed significant quantities of dipalmitoylglycerophosphocholines are produced. However, in the absence of CoA, the major species of phosphatidylcholine formed was 16:0–20:4. Stimulation of intact platelets with thrombin or ionophore A23187 caused a 3-fold to 10-fold increase in total acylation rates but there was a greater selection for arachidonate than palmitate under these conditions. The K_m for palmitoyllyso-GPC as the acceptor was 17 μM. These studies also revealed the CoA-dependent transacylase in rat platelet homogenates was unable to catalyze the esterification of lyso-phosphatidylcholine with free palmitic acid.

Reddy and Schmid[107] reported the addition of 0.5 mM CoA to microsomal preparations from dog heart increases the transfer of acyl groups from exogenous phosphatidylcholine to lyso-phosphatidylethanolamine but decreases the high selectivity for arachidonate that occurs when CoA is absent. Lyso-phosphatidylethanolamine and lyso-ethanolamine plasmalogens are equally good acyl acceptors in the reaction catalyzed by the CoA-independent transacylase but the lyso-plasmalogen is a much less effective acceptor than phosphatidylethanolamine when CoA is present. A bimodal pH optimum (7.5 and 4.5) was observed for the CoA-dependent transacylase with the rates being ca. 4–5 times higher at pH 4.5 than at pH 7.5. In contrast, the pH optimum range for the CoA-independent transacylase activity was 7.5–8.5 in identical microsomal preparations from dog heart. As observed in other systems, in the absence of CoA, the transfer of acyl groups from endogenous phospholipids to ^{14}C-labeled lyso-phosphatidylethanolamine produced more than 80% of the arachidonoyl species of phosphatidylethanolamine, whereas in the presence of CoA, the tetra-unsaturated species of acylated product was reduced from 83 to 63% of the total radioactivity in the ethanolamine phospholipids.[107] The results of these

experiments further emphasize the highest degree of selectivity (i.e. for 20:4) occurs with the CoA-independent transacylase and that the proportion of poylunsaturated acyl moieties is greatly reduced via CoA-dependent transacylation and even further decreased when acyl-CoAs are the acyl donors.

Microsomes isolated from dog heart have also been shown to catalyze the transfer of *sn*-2 acyl groups from phosphatidylcholine to lyso-phosphatidylserine in the presence of CoA. Optimal pH for this reaction occurs in the range of 4.5–5.0 where the transfer rates are about three times greater than at a neutral pH.[108] This CoA-dependent transacylase exhibits a preference for arachidonate over linoleate by a factor of 2.1, whereas the acyl-CoA acyltransferase in identical preparations exhibits a preference for linoleate over arachidonate by a factor of 3.7. The *sn*-2 acyl composition of phosphatidylserine is obviously controlled by both the CoA-dependent transacylase and by acyl-CoA acyltransferases.

The high selectivity of rabbit alveolar macrophages in their ability to acylate alkyl- and acyl-lysophospholipids with arachidonate prompted a detailed investigation of the acylation mechanism responsible.[115] These studies again demonstrated that the CoA-independent pathway accounted for the arachidonate specificity (see Section VI) observed in macrophages. In contrast, when CoA was present with 1-hexadecyl-2-lyso-GPC (K_m = 1.5 μM) as the acyl acceptor, the major molecular species of acylated product formed was 16:0–18:2. These results are in agreement with studies of the CoA-dependent transacylase in other cell types that indicate the CoA-induced acyl transfer is not as specific for arachidonate as for the CoA-independent transacylation reaction. The data obtained with macrophage membranes[115] differ from results obtained with platelet membranes[65] since, in the latter situation, the addition of CoA did not enhance the total extent of acylation. In fact, under the experimental conditions used, alkyllysoglycerophosphocholine did not even serve as a substrate for either the CoA-dependent or acyl-CoA-dependent reactions in the preparation of membranes from human platelets.[65]

Mouse thymocytes[139] and macrophages derived from mouse bone marrow[45] possess significant CoA-dependent transacylase activity. With thymocytes prelabeled with [^{14}C]arachidonic acid,[139] the direct CoA-mediated transfer of arachidonate only occurred with phosphatidylcholine as the donor and lyso-phosphatidylethanolamine as the acyl acceptor molecule. With different acyl donor molecules, the bone marrow-derived macrophages exhibited both a bidirectional and unidirectional transfer of arachidonate to the lyso-phospholipids by the CoA-dependent process.[45] The transfer of arachidonate from 1-acyl-2-arachidonoyl-GPC to lyso-phosphatidylethanolamine was shown to occur through a bidirectional movement, whereas a unidirectional transfer process was observed from 1-acyl-2-arachidonoyl-*sn*-glycero-3-phosphoinositol to lyso-phosphatidylcholine or lyso-phosphatidylethanolamine. In these experiments, the lysoplasmalogen analogs containing choline or ethanolamine were poorer acyl acceptor molecules than the corresponding acyllyso-phosphatides. The CoA-dependent system from macrophages was completely inhibited by sodium cholate, which is consistent with the concept that the acyl transfer via the reverse reaction catalyzed by the acyl-CoA-lyso-phospholipid acyltransferase.[45]

CoA-mediated transfer of linoleate has also been shown to take place between phosphatidylcholine and lyso-phosphatidylethanolamine in microsomes obtained from rat lung.[93] The K_m for CoA in this transacylation process with lyso-phosphatidylethanolamine as the acceptor was 1.5 μM. Other acyl acceptor molecules were lyso-phosphatidylglycerol and lyso-phosphatidylserine, but both were considerably less effective than lyso-phosphatidylethanolamine; lyso-phosphatidate was not an effective acceptor in the lung microsomal system.

Liver microsomes from rabbits possess a fairly high CoA-dependent transacylase activity, whereas they exhibit a very low cofactor-independent activity.[133] In the CoA-induced transacylation reaction, the K_m for CoA was 1.4 μM with 1-acyl-2-lyso-GPC as the acceptor (V_{max} = 2.7 nmol/min/mg protein) and 3.8 μM with 1-acyl-2-lyso-GPE as the acceptor (V_{max} = 1.2 nmol/min/mg protein). Specificity for the acyl transfer catalyzed by

the CoA-dependent transacylase indicated arachidonate and linoleate at the *sn*-2 position of phosphatidylcholine could be transferred to lyso-phosphatidylethanolamine in the presence of CoA, whereas the transfer of 16:0, 18:0, and 18:1 acyl moieties at the *sn*-2 position of phosphatidylcholine under the same conditions was negligible. An interesting finding in this work was that stearate at the *sn*-1 positon of either phosphatidylcholine or phosphatidylethanolamine could be transferred to lyso-phosphatidylethanolamine or lyso-phosphatidylcholine, respectively; the transfer of palmitate under identical conditions was extremely low. A comparison of the CoA-dependent and CoA-independent transacylase activities in various rabbit tissues indicate the CoA-dependent route is relatively high in lung > liver > testes > heart > kidney > brain > spleen > where the cofactor-independent pathway was highest in lung > heart > brain. The CoA-dependent transacylation catalyzed by liver microsomes was inhibited by oleic acid, linoeic acid, indomethacin, and octylglucoside in a similar fashion. Also, in comparison to acyl-CoA acyltransferases, the CoA-dependent transacylase is more sensitive to heat treatment, deoxycholate, chlorpromazine, dibucaine, and tetracaine.[133]

CoA-dependent transacylation by rat brain microsomes exhibited a preference for acceptors in the order of lyso-phosphatidylcholine > lyso-phosphatidylethanolamine ≫ lyso-plasmenylethanolamine.[29] Although completely different from the CoA-independent transacylase with respect to acyl transfer specificity (the latter selective for 22:6 and 20:4), the CoA-dependent enzyme was similar to acyl-CoA acyltransferase in transferring unsaturated acyl groupings to the same acceptor molecule. Certain affinities seen in the acyl transfer process were dependent on the acceptor used. Lyso-plasmenylethanolamine only esterified oleate in the presence of CoA, whereas lyso-phosphatidylethanolamine and lyso-phosphatidylcholine were good acceptors for both arachidonate and oleate in the presence of CoA. Furthermore, significant quantities of palmitate were transferred when lyso-phosphatidylcholine was the acyl acceptor, but this was not observed in rat brain microsomes with lyso-phosphatidylethanolamine as the acceptor molecule.

Tumor cells also contain active transacylase systems. Recent work from our laboratory examined factors that influence CoA-dependent transacylation, CoA-independent transacylation (see Section VI), and acyl-CoA-dependent acylations using 1-hexadecyl-2-lyso-GPC and 1-hexadecyl-2-lyso-GPE as the acyl acceptors.[69] As with other systems, the CoA-independent pathway produced the highest percentage of polyunsaturated acylated products, the CoA-dependent system formed an intermediate range of molecular species, and the acyl-CoA system yielded products with the least degree of unsaturation.

The molecular remodeling of phosphatidylinositol has been investigated using rat liver microsomes.[38] Acylation of 2-lyso-*sn*-phosphatidylinositol occurred in a CoA-dependent (ATP-independent) manner with optimum activity reached at pH 7.5; the transacylase required no divalent cations. Zn^{2+} strongly inhibits the enzyme, whereas Ca^{2+} or Mg^{2+} have no effect. K_m values for CoA and the 2-lyso *sn*-phosphatidylinositol substrate were 14 and 30 μM, respectively. Stearate was the major acyl group esterified at the *sn*-1 position by the CoA-dependent transacylase to form phosphatidylinositol; however, the endogenous source of the stearate was not determined. Specificity of this CoA-dependent transacylase for stearate is compatible with what would be expected for unique acyl composition of phosphatidylinositol (i.e. stearate at the *sn*-1 position and arachidonate at the *sn*-2 position). The arachidonate is incorporated into the *sn*-2 position of phosphatidylinositol by an acyl-CoA acyltransferase.

VI. CoA-INDEPENDENT TRANSACYLATION REACTIONS

A. *Lyso-phosphatidylcholine (lyso-PC):Lyso-phosphatidylcholine Transacylase (Lyso-phospholipase)*

The first evidence for the presence of a CoA-independent transacylation involving phospholipids was reported for a cytosolic system isolated from rat liver by Marinetti *et al.*[74] By using double labeled 1-[^{14}C]acyl-2-lyso-*sn*-glycero-3-[^{31}P]phosphocholine, the

FIG. 3. Reaction catalyzed by lyso-phosphatidylcholine:lyso-phosphatidylcholine CoA-independent transacylase.

reaction sequence was shown to take between two molecules of lyso-phosphatidylcholine (Fig. 3).[43,142] The transfer of an acyl group from one lyso-phospholipid to another appears to occur in two steps. In the initial step, one molecule of lyso-phosphatidylcholine is hydrolyzed to form glycerophosphocholine and an acyl–enzyme covalent intermediate. The second step appears to involve the binding of the second molecule of lyso-phosphatidylcholine to the acyl-enzyme complex where the hydroxyl moiety at the *sn*-2 position of the lyso-lipid acts as a nucleophile to produce phosphatidylcholine; alternately, the acyl–enzyme complex can be deacylated to produce a free fatty acid. Free fatty acids cannot be esterified to lyso-phosphatidylcholine by the transacylase.

Lyso-phosphatidylcholine:lyso-phosphatidylcholine transacylase (lyso-phospholipase) has been purified to near homogeneity from the lung of rats[19] and rabbits[23] and from rabbit heart[49,50] and bovine[118] heart. These enzyme purification studies indicate a single protein is responsible for the entire transacylation sequence since all of the enzyme activities responsible for catalyzing the hydrolytic and transfer reaction steps copurify. Mass spectrographic studies with $H_2{}^{18}O$[145] and the purified transacylase revealed the ^{18}O is only incorporated into the palmitate released to the water but none is found in the phosphatidylcholine product formed in the transacylation process. These data are consistent with the concept that the transacylation between two lyso-phosphatidylcholine molecules is initiated by hydrolytic cleavage of the acyl moiety of the lyso-phospholipid and supports the concept of a covalent acyl-enzyme intermediate being formed in the overall reaction.

Partial purification of the lyso-PC:lyso-PC transacylase from rat lung was first reported by Abe *et al.*,[1] both the acyl transfer and hydrolytic enzymes copurified at all stages of chromatography. Although their 100-fold purification in 30% yield contained three main protein bands after polyacrylamide gel electrophoresis (pH 8.3), the enzyme preparation provided useful information about the catalytic properties of the dual activities associated with it. The two activities exhibited similar responses with regard to stability, identical K_ms (100 μM) for lyso-phosphatidylcholine, and the same pH activity curves with an optimum pH at 6.0–6.5. No divalent cations were required and deoxycholate was strongly inhibitory.

The cytosolic lyso-PC:lyso-PC transacylase purified (~250-fold) to homogeneity from rat lung gave a single band on polyacrylamide disc gel electrophoresis and had an estimated molecular weight of 50,000.[19] Free fatty acids, CoA, and ATP did not influence the formation of phosphatidylcholine by the purified enzyme. Interestingly, not all lyso-phospholipases possess transacylase activity; for example, purified lyso-phospholipases from pancreas and liver of beef catalyze the hydrolysis of fatty acids from lyso-phosphatidylcholine, but they do not transfer the acyl moiety to form phosphatidylcholine.[19] Support for the conclusion that the highly purified pulmonary lyso-PC:lyso-PC transacylase activity is a single bifunctional protein is based on the following observations: (1) the ratio of lyso-phospholipase/transacylase activity remained constant at each step during the 250-fold purification procedure and the purified enzyme behaved as a single component on polyacrylamide gels, (2) both reactions exhibited similar kinetics with respect to variations in protein concentration and time, and (3) *sn*-2-deoxy analog of phosphatidylcholine is a substrate for the deacylation step but not the transfer reaction and, when the deoxy analog is mixed equimolar with the lyso-phosphatidylcholine, the amount of product (phosphatidylcholine) formed is reduced by one-half. If a separate enzyme was responsible for the acyl transfer reaction, one would not expect the deoxy-phosphatidylcholine to have any effect on transacylation. The ratio of lyso-phospholipase to transacylase activity in the purified lung enzyme preparation varied depending on the concentration of substrate with the level of transacylase protein and took on greater importance with higher concentrations of lyso-phosphatidylcholine. Also, this investigation[19] indicated

phosphatidylcholine suppresses the transacylase activity through product inhibition. In other studies, Perez-Gil *et al.*[98] found albumin also inhibited the transacylation step but it did not affect hydrolysis of the acyl group; the apparent reason for these results is that albumin alters the critical micellar concentration of the substrate.

Experiments by Vianen and van den Bosch[148] with the purified enzyme from rat lung confirmed only a single protein band was observed on both normal and sodium dodecyl sulfate polyacrylamide disc gels following electrophoresis. Also heat treatment and exposure to diisopropylfluorophosphate caused a parallel loss of both the hydrolytic and acyl transfer activities. Data obtained about the substrate specificity of the pulmonary transacylase indicated both lyso-phosphatidylethanolamine and lyso-phosphatidylglycerol could be converted to their corresponding acylated products whereas acylglycerols, cholesterol, *sn*-glycero-3-P, and *sn*-glycero-3-phosphocholine did not serve as acyl acceptors. With lyso-phosphatidylcholine as the substrate, preference was observed for the palmitoyl species over those containing a stearoyl moiety.[148] Lyso-phosphatidylcholines with acyl chains consisting of >10 carbon atoms served as acyl acceptors.[144] These experiments also demonstrated the enzyme from rat lung could hydrolyze the acyl group when the substrate existed as monomers, whereas the transacylation reaction only took place if the substrate was present in a miceller form. Also, if the lyso-phosphatidylcholine substrate was embedded in liposomes prepared from total lipids of rat lung, transacylation did not occur.[144]

Casals *et al.*[22] found the transacylase exhibited a greater selectivity for lyso-phosphatidylcholine as an acyl acceptor than as an acyl donor. Both the transacylase and hydrolytic activities preferred lyso-phosphatidylcholine over lyso-phosphatidylethanolamine as a substrate; also, in substrate competition experiments, increasing the concentration of lyso-phosphatidylethanolamine in a micellar mixture with lyso-phosphatidylcholine did not affect the rate of phosphatidylcholine formation by the transacylase. In these experiments, the enzyme exhibited a preference for saturated acyl chains in the transacylation step, whereas the lyso-phospholipase activity appeared to utilize substrates independent of the degree of unsaturation.[22]

The lyso-PC:lyso-PC transacylase from rabbit lung was purified 80-fold with a constant hydrolysis/acylation ratio of two during all purification steps.[23] A single band was obtained when the purified enzyme was analyzed by polyacrylamide gel electrophoresis. These results indicated one polypeptide is responsible for both the acyl hydrolysis and acyl transfer; the molecular weight of the peptide was in the range of 58–70 kDa and the amino acid composition had an acidic character. These studies further documented the physical state of the substrate is very important in determining which function of the enzyme activity is expressed. In agreement with van Heusden *et al.*,[144] Casals and coworkers[23] found ($<$CMC) the substrate in the monomeric form is hydrolyzed to the free acid, whereas the micellar form of the substrate promotes the transacylation component to form phosphatidylcholine. The loss of enzyme activity after treatment with iodoacetate and the protective action of β-mercaptoethanol indicates the presence of essential thiol groups in the enzyme. As with the rat enzyme, the activities of the rabbit lung enzyme for both the hydrolysis and transacylation of the acyl moiety are inactivated in parallel by diisopropylfluorophosphate or by increasing temperatures. Also, lyophilization, freezing/thawing, and detergents decrease both activities. The transacylase activity is very sensitive to ionic strength, with the activity steadily increasing with the ionic strength (up to 0.125 M NaCl); these results indicate the pure form of enzymes requires salts for optimum transacylase activity. The K_m was smaller for the hydrolytic activity (10.7 μM) than the transacylase activity (33.8 μM), whereas the V_{max} (nmol/min/mg protein) was 30.3 and 15.2, respectively. Since the activation energy for the two reactions were found to be similar, it was concluded the limiting step would be the initial acylation of the enzyme which is the common intermediary complex for both reactions.[23]

In solutions of high ionic strength, the lyso-phosphatidylcholine:lyso-phosphatidylcholine transacylase has a relatively disordered conformation.[4] As the salt concentration is decreased, the protein exhibits a more ordered structure and the transacylase activity

decreases without any influence on the hydrolytic step. Acidic denaturation of the enzyme revealed ionizable groups with a p*K* of 5.9–6.4; the protein was stable in alkaline pH. Addition of lipids to the purified enzyme mimicked the results obtained with NaCl addition, causing a stabilization of the disordered form. The conclusion reached from this work is the lyso-PC:lyso-PC transacylase can exist in two conformational forms, each exhibiting a difference in their ability to bind the lyso-phosphatidylcholine substrate. Based on the conformational properties and the amino acid composition of the enzyme, Arche *et al.*[4] have suggested the transacylase is a peripheral membrane protein. In a separate investigation, Casals *et al.*[24] determined the addition of lipids did not cause any change in the protein structure of the enzyme based on circular dichroism spectra. The authors hypothesized the lipids affect the hydrolysis/transacylation ratio (i.e. lipid addition causes a decrease in this ratio) by influencing the physical state of the substrate and probably the interaction of the enzyme with the substrate.

The pH-dependent inactivation of transacylase activity was investigated using ultrasonic cavitation. Two first order inactivation constants were observed with a pH dependency and a transition between them having a p*K* of 5.9.[102] These results indicated the conformational changes in the enzyme would alter the accessibility of the active site to the substrate.

A purified preparation of lyso-phosphatidylcholine:lyso-phosphatidylcholine transacylase/lyso-phospholipase from rabbit lung was shown to be inactivated by diethylpyrocarbonate; this reagent reacts with several amino acid chains but it is highly specific for histidine below pH 7.0.[99] The loss in enzyme activity by diethylpyrocarbonate could be partially recovered after hydroxylamine treatment and both lyso-phosphatidylcholine and lyso-phosphatidylethanolamine could partially protect the transacylase against inactivation. The inactivation rate for the transacylase/lyso-phospholipase activity by diethylpyrocarbonate is pH dependent and involves an ionizable group with a p*K* of 6.5. The increased absorption at a wavelength of 242 nm following treatment of the transacylase with diethylpyrocarbonate indicated histidine residues in the enzyme were altered. Results of this work suggested two histidine residues are necessary for the hydrolytic reaction and one histidine residue for the transacylation and these residues would appear to be at or very close to the active site of the enzyme.

Perez-Gil and coworkers[100] have also conducted other experiments using chemical modification approaches to assess the essential features of the active site of the transacylase. Diisopropylfluorophosphate inhibits both the hydrolytic and transacylation components of the lyso-PC:lyso-PC transacylase.[100] This inhibition was not protected by increased levels of substrate and the treated enzyme exhibited a more ordered protein arrangement than the native enzyme, based on the circular dichroism spectrum. These findings and the fact another serine inhibitor (phenylmethanesulfonyl fluoride) did not influence either hydrolysis or the transacylation steps indicate that serine residues are not involved in binding of the substrate by the transacylase. In other experiments, the inhibition of the enzyme by sulfhydryl reagents (*N*-ethylmaleimide and *p*-hydroxymercury benzoate), the kinetics of inhibition, and protection against this inhibition by the substrate indicated two essential cysteine residues are located at the active site. The reduced activity of the enzyme after sulfhydryl modification was not associated with any significant change in the protein confirmation of the transacylase. Both the hydrolytic and transacylation activities of the transacylase were also inhibited by tetranitromethane, a tyrosine inhibitor and there was partial protection of both catalytic activities by the substrate; these data are in support of essential tyrosines being at or near the active site of the enzyme. In addition, a reagent that reacts with the protonated form of carboxylate (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline at pH 5.0) inhibited the hydrolytic activity but did not affect the transacylation component. Moreover, this inhibition could also be prevented by the substrate. Thus, these findings are consistent with the mechanism that a carboxyl grouping at the active site also participates in the hydrolytic step catalyzed by the lyso-PC:lyso-PC transacylase.[100]

Martin *et al.*[75] proposed a theoretical model for the lyso-phosphatidylcholine:lyso-phosphatidylcholine transacylase based on (1) an acyl transfer in a dual substrate reaction for

TABLE 2. Different Acylation Mechanisms involving Lyso-Phospholipids as Acyl Acceptors Produce Different Molecular Species of Phospholipid Products

Enzyme	Donor lipid	Type of molecular species of phospholipid formed
Acyl-CoA acyltransferase	Acyl-CoA	Least degree of unsaturation (usually saturated and monoenoic acyl species)
CoA-dependent transacylase	Intact phospholipid	Generally an intermediate degree of unsaturation (dienoic and some tetraenoic species)
CoA-independent transacylase	Lyso-phosphatidylcholine	Disaturated species of phosphatidylcholine
	Intact phospholipid	Primarily arachidonoyl and higher polyenoic species

identical substrates, (2) the substrate is an amphiphilic molecule that can exist in two physical forms, and (3) the reaction mechanism precedes via a covalent-linked acyl–enzyme complex as an intermediate that can also react with water in a secondary hydrolytic reaction. Proposed kinetic equations, based on steady-state theory, developed for this model gave theoretical plots that were in agreement with the experimentally obtained kinetic data, including those found for inhibition by reaction products. The authors believed that the data showing differences in the kinetic patterns for the dual catalytic activities of the enzyme indicate competition occurs between two nucleophiles for the covalent acyl–enzyme complex rather than the different enzyme actions being due to the physical state of the substrate. Based on this kinetic model, Perez-Gil *et al.*[101] determined the pK values for the free enzyme and the substrate–enzyme complex. These results indicated catalysis was dependent on the deprotonation of the histidine residue with a pK of ca. 5.7. The very high and exothermic enthalpy measured indicated a conformational modification of the enzyme is associated with the ionization of the essential histidine component.

At one point, it was thought that lyso-phosphatidylcholine: lyso-phosphatidylcholine transacylase possibly could play an important role in the production of pulmonary surfactant, i.e. dipalmitoylglycerophosphocholine. However, studies with adult[10] and fetal[41] alveolar type II cells, adult rat lungs,[143,146,147] developing rat lung,[25] and rabbit and mouse lungs[146] indicated the acyl-CoA acyltransferase pathway, instead of the transacylase route, was the main contributor to the synthesis of pulmonary surfactant. Surprisingly, 100,000 *g* supernatants prepared from lungs of pig, bovine, horse, and sheep contained no detectable lyso-phosphatidylcholine: lyso-phosphatidylcholine transacylase activity. Thus, there is overwhelming evidence to indicate the transacylation reaction between two 1-palmitoyl-2-lyso-GPCs is probably of trivial importance in the production of the disaturated species of pulmonary surfactant.

A lyso-phospholipase/transacylase activity has also been purified to near homogeneity from both rabbit myocardium[49] and bovine heart.[118] The enzyme from rabbit had a molecular weight of 63,000 compared to 128,000 for the bovine preparation. Data obtained for a partially purified lyso-phospholipase/transacylase from the cytosolic fraction of rabbit heart revealed a V_{max} of 9.5 nmol/min/mg protein and a K_m of 7.5 μM.[50] However, the transacylation activity of the rabbit enzyme was not prominent at submicellar concentrations (2 μM) of lyso-phosphatidylcholine.[49] Kinetic plots done with the purified enzyme indicated the reaction mechanism involved two molecules of substrate forming a ternary complex with the enzyme. Inhibition of the lyso-PC: lyso-PC transacylase by L-palmitoylcarnitine could be reversed by dilution or dialysis. The authors suggested the inhibition of transacylase activity by L-palmitoylcarnitine might be of pathological relevance since acylcarnitines are elevated in ischemic myocardium and could perpetuate the accumulation of lyso-phospholipids during ischemia.[49]

Interestingly, lyso-phospholipase activity, but no transacylase activity, could be detected in rat cardiac myocytes and rat mesenchymal cells grown in culture.[48] In contrast, rabbit myocytes obtained by perfusion of isolated hearts with collagenase did contain both lyso-phospholipase and transacylase activities. As with studies of the lung enzyme, these data point out significant species differences in the catalytic properties associated with lyso-PC: lyso-PC transacylase.

Unlike the enzyme from rat or rabbit lung and rabbit heart, the lyso-phosphatidylcholine:lyso-phosphatidylcholine transacylase from bovine heart is a microsomal rather than cytosolic protein.[118] It has recently been solubilized and purified almost to homogeneity, and its properties have been characterized.[118] Enzyme activity was dependent on either CoA, acetyl-CoA, or oleoyl-CoA albeit not as substrates, whereas palmitoyl-CoA, stearoyl-CoA, arachidonic acid, oleic acid, phosphatidylethanolamine, and phosphatidylserine were ineffective as activators. On the other hand, both palmitic acid and phosphatidylcholine were able to activate the transacylase further in the presence of oleoyl-CoA. The purified transacylase from bovine heart did not possess any acyl-CoA:lyso-phosphatidylcholine acyltransferase, lyso-phospholipase, or acyl-CoA hydrolase activities. Furthermore, the enzyme activity exhibited a high degree of substrate selectivity since the acyl moiety of lyso-phosphatidylcholine could not be transferred to lyso-phosphatidylethanolamine, lyso-phosphatidylinositol, or lyso-phosphatidylserine. Selectivity for lyso-phosphatidylcholine was independent of the extent of unsaturation in the acyl side chain.[118] The membrane location, lack of lyso-phospholipase activity, and the selective substrate specificity of this transacylase distinguishes it from those found in the cytosolic fraction.

A cytosolic lyso-PC:lyso-PC transacylase activity similar in properties to those described for the rat lung enzyme has also been reported in rat gastric mucosa.[71] Both the hydrolytic and acyl transfer activities of the gastric enzyme maintained the same ratio throughout all purification steps and after isoelectrofocusing. The transacylase and lyso-phospholipase activities associated with the partially purified enzyme preparation had identical K_m values (0.25 mM) for lyso-phosphatidylcholine, and exhibited an optimum pH of 6.0; both the hydrolytic and acylation activities responded in the same manner to Ca^{2+} or Mg^{2+} (no effect), detergents (both taurocholate and Triton X-100 stimulated activity), stability to storage, and heat treatment. The authors suggested the role of the lyso-PC:lyso-PC transacylase in the gastric mucosa could be important in preventing the build-up of lyso-phospholipids to toxic levels during the gastrointestinal absorption of fat. Also, under conditions such as high substrate concentrations or when the ATP supply is limited, the transacylation mechanism could provide an alternate source of phosphatidylcholine synthesis.[71]

B. *Diacyl phospholipids:Lyso-phospholipid Transacylases*

1. *Choline- and Ethanolamine-containing Lyso-glycerophosphatides as Acyl Acceptors*

A variety of reports can be found in the literature for a CoA-independent transacylase activity that is responsible for catalyzing the transfer of an acyl moiety from a diacyl glycerophosphatide (typically phosphatidylcholine) to a lyso-phospholipid acceptor molecule, generally containing either choline or ethanolamine (Fig. 4). The acyl acceptor molecule can have an acyl, alkyl, or alk-1-enyl aliphatic chain at the *sn*-1 position of the lyso-phospholipid. Perhaps the first report of this type of CoA-independent reaction was observed in studies of the enzymatic synthesis of ethanolamine plasmalogens.[97,158] These plasmalogen experiments relied on an enzymatic activity associated with microsomal preparations (containing no acyl-CoAs or CoA) to acylate radiolabeled 1-alkyl-2-lyso-GPE to 1-alkyl-2-acyl-GPE, the immediate precursor utilized by a Δ^1-desaturase to produce ethanolamine plasmalogens. Since the acylation of the lyso-phospholipid in this system was only a means to generate the formation of the plasmalogen precursor, the enzymatic activity responsible for the acyl transfer was not characterized, other than the fact no cofactors were required.

FIG. 4. Typical reaction catalyzed by an intact phospholipid:lyso-phospholipid CoA-independent transacylase.

Later Kramer and Deykin[63] described a crude membrane fraction from human platelets that could catalyze the transfer of arachidonate from phosphatidylcholine to lyso-plasmenyl-ethanolamine without any requirement for CoA. The transacylation was optimal at pH 7–8. Free arachidonic acid did not influence the activity, but it could be inhibited by *N*-ethylmaleimide and Triton X-100. The rates of acyl transfer from phosphatidylcholine to the acyl acceptor were arachidonate > eicosatrienoate > oleate. The preference for acyl acceptors was lyso-plasmenylethanolamine > lyso-phosphatidylethanolamine > lyso-phosphatidylserine; lyso-phosphatidylinositol did not participate as a substrate.[63] In a separate report from the same laboratory, it was shown that lyso-PAF (1-alkyl-2-lyso-GPC) is also acylated exclusively with arachidonate derived from phosphatidylcholine by the CoA-independent transacylase.[64] These findings indicated the CoA-independent transacylation reaction is responsible for the enrichment of arachidonate in the ether-linked phospholipids found in platelets.

Also, with platelet lysates from rats, the CoA-independent transacylase was shown to promote the synthesis of the 16:0–20:4 species of phosphatidylcholine as the major product when 1-[^{14}C]palmitoyl-2-lyso-GPC was the acyl acceptor.[76] The transfer of linoleate also occurs via this CoA-independent mechanism, but the rate is four times less than with arachidonate, and oleate is transferred to only a minimal extent. Inclusion of CoA in the incubations of platelet lysates from rats induced the formation of 16:0–16:0 species of phosphatidylcholine equal to the amount of 16:0–20:4 species produced.

Robinson and coworkers[115] used 1-[^{3}H]hexadecyl-2-lyso-GPC to investigate the substrate specificities involving three acylation mechanisms (CoA-dependent, CoA-independent, and acyl-CoA-dependent) in rabbit alveolar macrophages since it was found that the kinetics for the incorporation of arachidonic acid into phospholipids of intact cells differed from other fatty acids. Kinetic data obtained with intact cells in the formation of the arachidonoyl species of phosphatidylcholine when 1-[^{14}C]palmitoyl-2-lyso-GPC was used as the acyl acceptor were similar to those found with [1-^{3}H]hexadecyl-2-lyso-GPC. The results of this work demonstrated the CoA-independent transacylase was responsible for the selective esterification of lyso-phospholipids with endogenous arachidonate and that the reaction catalyzed was independent of any free fatty acids in membrane preparations. The CoA-dependent mechanism in these experiments produced molecular species of alkyl choline-containing glycero-phospholipids of intermediary unsaturation, with the 16:0–18:2 species representing the largest proportion. Even more striking was the acyl-CoA acyltransferase formed a product having the least amount of unsaturated acyl moieties with 16:0–18:2, 16:0–18:1, and 16:0–16:0 species being the prominent products. The molecular species formed (mainly 20:4) by the CoA-independent transacylase most closely resembled the molecular species of alkylacyglycerophosphocholines obtained in the acylation of the lyso-phospholipid by the intact macrophages. K_m values for the CoA-independent, CoA-dependent, and acyl-CoA-dependent acylation reactions in microsomes isolated from the rabbit macrophages were 1.1, 1.5, and 2.5 μM for 1-[^{3}H]-hexadecyl-2-lyso-GPC, respectively; the velocity of the reaction in the presence of CoA, ATP, and Mg^{2+} was equal to the sum of the three different acylation reactions measured, with the CoA-independent transacylase possessing the highest substrate affinity and the major velocity component at low substrate concentrations.

In related work with microsomal preparations from rabbit alveolar macrophages,[134] it was demonstrated that endogenous phosphatidylcholine was the major source of the arachidonate transferred to 1-alkyl-2-lyso-GPC in the CoA-independent acylation reaction; although transfer of arachidonate from phosphatidylethanolamine to the acyl acceptor also occurred, the rate was much lower than when phosphatidylcholine was the donor. Addition of CoA or mepacrine had little effect on the transacylation, whereas 0.02% Triton and 0.2% cholate were strongly inhibitory. Results obtained with intact rabbit platelets[73] were similar in that the tetraenoic species of alkylacylglycerophosphocholines were shown to contain ca. 80% of the radioactivity when either tritiated lyso-PAF or PAF were metabolized by the platelets; in both instances, phosphatidylcholine appeared to be the source of arachidonate in the reacylation process.

The CoA-independent transacylase has also been shown to be highly selective for docosahexaenoate by microsomal preparations from Ehrlich ascites cells;[77] arachidonate was transacylated but to a significantly lesser extent than the hexaenoic species. In the absence of CoA, only ether-linked lyso-phospholipids (1-alkyl-2-lyso-GPC and 1-alkyl-2-lyso-GPE) served as acyl acceptors. Lyso-phosphatidylcholine and lyso-phosphatidylethanolamine could be acylated only when acyl-CoAs were added to the incubations containing Ehrlich ascites microsomes.

The rate of CoA-independent transacylation in rat brain microsomes for lyso-plasmenylethanolamine is approximately twice as high as lyso-phosphatidylethanolamine or lyso-phosphatidylcholine.[79] Moreover, the molecular species of phospholipid product formed by the CoA-independent transacylase were exclusively esterified with docosahexaenoate and arachidonate regardless of the lyso-phospholipid acceptor used. It is thought the cofactor-independent acylation system is responsible for the high levels of docosahexaenoate found in the ethanolamine plasmalogens of brain.

Sugiura and Waku[134] also showed that the CoA-independent transacylase plays a significant role in the regulation of the metabolism of docosahexaenoic acid (22:6ω3) in rabbit alveolar macrophages. [^{14}C]labeled docoahexaenoate was transferred from exogenously added phosphatidylcholine to choline- and ethanolamine-containing ether-linked lyso-phospholipids.[132] With 1-alkyl-2-lyso-GPC as the acyl acceptor, the rates of 22:6 and 20:4 transfer were comparable, whereas when 1-alk-1-enyl-2-lyso-GPE was used as the acceptor the transfer of 22:6 was twice that of arachidonate. Similar findings were reported by Ojima *et al.*[94] for the transacylation of 1-alkyl-2-lyso-GPE with docosahexaenoate and arachidonate by a CoA-independent transacylase in rat brain microsomes.

Subsequent experiments by Sugiura *et al.*[131] were done to characterize the selectivity of both donor and acceptor molecules by the CoA-independent transacylase in microsomes isolated from rabbit alveolar macrophages. [^{14}C]labeled 20:4, 20:5, 22:4, and 22:6 acyl moieties at the *sn*-2 position of phosphatidylcholine exhibited essentially equally good transfer rates to 1-alkyl-2-lyso-GPC in the absence of any added cofactors or when CoA was present; on the other hand, the transfer rates for 16:0, 18:0, and 18:1 from phosphatidylcholine to the alkyl acceptor were very low under identical conditions. In contrast to those findings, the transfer of arachidonate from phosphatidylethanolamine and phosphatidylinositol as the donor molecules to 1-alkyl-2-lyso-GPC or to 1-acyl-2-lyso-GPC was dramatically increased when CoA was present. This work further emphasizes that the CoA-independent transacylase exhibits distinct substrate specificities for both the donor and acceptor molecules involved in the transacylation reactions.

Microsomes isolated from dog heart have been shown to be capable of transferring arachidonate from both exogenous[106,107] and endogenous[107] phosphatidylcholine to either lyso-phosphatidylethanolamine or lyso-plasmenylethanolamine. The acyl analog was only slightly better as an acyl acceptor than lyso-plasmalogen in the absence of CoA, but the former became ca. 10-fold more effective as an acyl acceptor when CoA was added to the microsomal incubations. The pH optimum for the CoA-independent transacylation in dog heart is 7.5–8.5. In this sytem, the transfer of endogenous acyl moieties to 1-[^{14}C]palmitoyl-2-lyso-GPE results in more than 80% of the tetraenoic species of phosphatidylethanolamine being produced.

Rabbit liver microsomes possess a relatively low CoA-independent transacylase activity compared to membrane fractions from lung > heart > brain.[133] However, good acyl (18:2 and 20:4) transfer rates were observed with liver microsomes in the presence of CoA with either phosphatidylcholine or phosphatidylethanolamine as the acyl donors and lyso-phosphatidylethanolamine and lyso-phosphatidylcholine as the acyl acceptors.

Winkler *et al.*[156] have characterized the CoA-independent transacylase activity in U937 cells, a human monocytic cell line. Microsomal preparations from these cells were the source of the enzyme activity that selectively transferred arachidonate to 1-alkyl-2-lyso-GPC ($K_m = 0.4\,\mu$M) as the acyl acceptor, with the optimum pH (6.5–9.0) exhibiting a wide range. The expressed enzyme activity did not require Ca^{2+}, Mg^{2+}, CoA, or

ATP and was heat sensitive. Dimethylsulfoxide-induced differentiation of the U937 cells into a macrophage form and dexamethasone treatment did not influence the transacylation rate. Serine esterase inhibitors (phenylmethylsulfonylfluoride, *N*-tosyl-L-phenylalanine, chloromethylketone) and a histidine inhibitor (diethyl pyrocarbonate) inhibited the CoA-independent transacylase in the membrane preparations from the U937 cells. These latter results are consistent with the characteristic properties of the well-known LCAT (lecithin cholesterol acyltransferase) enzyme. The transacylase activity was not affected by common phospholipase A_2 inhibitors (quinacrine, aristolochic acid, and arachidonic acid) which is in agreement with other published studies that indicate the properties of the CoA-independent transacylase are distinctly different from those generally associated with the low molecular weight phospholipase A_2 enzymes (see Ref. 156 for summary). Sensitivity of this transacylase to acid, stability to 10 mM 2-mercaptoethanol, and inhibition by detergents further confirm the activity completely differs in its properties from known phospholipase A_2 activities.[156]

2. N-*Acylation Reactions*

N-acylethanolamine phospholipids accumulate in infarcted but not normal canine heart. A number of reports by Schmid and his coworkers[89,90,105,109,110,121,122] have documented these *N*-acylethanolamine phospholipids are synthesized by a membrane-bound CoA-independent transacylase (*N*-acyltransferase) that requires millimolar concentrations of Ca^{2+}. The enzyme activity responsible for *N*-acylation has been described for cell free preparations from brain[90] and heart[89,109,110,121,122] of normal dogs. The *N*-acyltransacylase is heat sensitive and inhibited by sulfhydryl reagents. At pH 8.0, Sr^{2+}, Mn^{2+}, and Ba^{2+} can replace Ca^{2+} as a divalent cation requirement, whereas other divalent cations are less effective. Triton X-100 had no effect on the transacylase activity; however, trypsin in combination with Triton X-100 reduced the transacylase activity to 30% of the control value, compared to a reduction of *N*-acylation by trypsin alone to only 60% of the controls.[121]

Phosphatidylcholine,[110,121] 1-acyl-2-lyso-GPC,[121] and cardiolipin[110] can serve as acyl donors in the *N*-acylation reaction. Studies by Reddy *et al.*[105] with labeled 1,2-dipalmitoyl and 1-palmitoyl-2-linoleoyl species of phosphatidylcholine indicated the acyl transfer is via an intramolecular transacylation from 1-*O*-acyl to *N*-acyl groups of phosphatidylethanolamine which means a primary hydroxyl group at the *sn*-1 position also becomes available on phosphatidylethanolamine to serve as an additional acyl acceptor during *N*-acylation. In experiments with oxygen-18 labeled water, none of the ^{18}O was incorporated into either the amide or *O*-acyl moieties which supports the notion of an acyl transfer without hydrolysis.[122] From these results, the authors concluded a covalently-linked acyl–enzyme complex is formed as an intermediate during the *N*-acylation process.

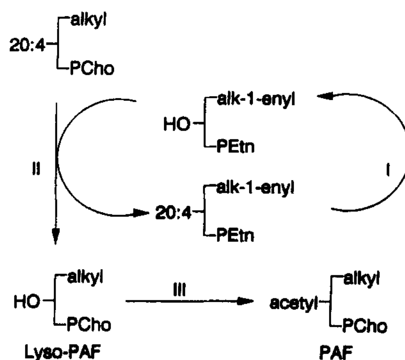

FIG. 5. Reactions illustrating the involvement of a CoA-independent transacylase in the formation of PAF via the remodeling pathway of biosynthesis. The individual reactions are catalyzed by the following enzymes: I—phospholipase A_2, II—a CoA-independent transacylase, and III—acetyl-CoA: lyso-PAF acetyltransferase.

VII. THE INVOLVEMENT OF A CoA-INDEPENDENT TRANSACYLASE IN THE BIOSYNTHESIS OF PAF

The possibility that a transacylase may trigger the biosynthesis of PAF (Fig. 5) was first suggested by the experiments reported by Sugiura *et al.*[129] in that, when 200 μM alk-1-enyl(alkyl)lyso-GPE (10–15% as alkyl) is added to PMNs, the amounts of PAF produced is increased to levels comparable to those induced by opsonized zymosan-stimulated PMNs (4.5 pmol/30 min/2.5 $\times$ 10^6 cells). The addition of alk-1-enyl(alkyl)lyso-GPE to cells caused the hydrolysis of alkylacyl-GPC to generate lyso-PAF, presumably mediated by CoA-independent transacylation, and thus leads to the formation of PAF. These results are further substantiated by the studies of Tessner *et al.*[138], Nieto *et al.*[91] and Uemura *et al.*[141] Tessner *et al.*[138] observed that arachidonoyl-containing alk-1-enylacyl-GPE is hydrolyzed during stimulation of human neutrophils by ionophore A23187 with concomitant accumulation of alk-1-enyllyso-GPE, a substrate required for any transacylation reaction. Both Nieto *et al.*[91] and Uemura *et al.*[141] have shown [^{3}H]alkyllyso-GPC (lyso-PAF) is liberated when alkyllyso-GPC, acyllyso-GPC, and alk-1-enyllyso-GPE is added to membranes prelabeled with [^{3}H]alkylacyl-GPC in human neutrophils and HL-60 cells, respectively. In addition, Uemura *et al.*[141] demonstrated acyllyso-GPE and alkyllyso-GPE are also able to stimulate the formation of [^{3}H]alkyllyso-GPC from [^{3}H]alkylacyl-GPC precursor pool associated with HL-60 cell membranes, whereas other glycerolipids containing free hydroxyl groups (D-lyso-PAF, lysophosphatidylserine, lysophosphatidylinositol, diacylglycerols, alkylglycerols, and monoacylglycerols), cholesterol, phosphatidylcholine and phosphatidylethanolamine are inactive under identical incubation conditions. Consistent with the above findings, Uemura *et al.*[141] were able to show [^{3}H]arachidonate could be transferred from alkyl-[^{3}H]arachidonoyl-GPC to alk-1-enyllyso-GPE to form alk-1-enyl-[^{3}H]arachidonoyl-GPE in HL-60 cell membrane fractions. This observed CoA-independent transacylase reaction is tightly coupled to PAF production, since in the presence of acetyl-CoA the addition of alk-1-enyllyso-GPE to membrane preparations isolated from HL-60 cells or human neutrophils stimulated the formation of PAF.[91,141] This transacylase activity is not affected by Ca^{2+}, EGTA, or a known phospholipase A_2 inhibitor, *p*-bromophenacylbromide. Thus, evidence from both *in vivo* and *in vitro* experiments indicate that a CoA-independent transacylase can be responsible for the formation of the lyso-PAF intermediate in the remodeling route of PAF biosynthesis.

VIII. CONCLUSIONS

It is clear from the many studies described in this review that the CoA-dependent and CoA-independent transacylases have a major role in the remodeling of membrane phospholipids; the different substrate specificities of these two types of transacylases makes their function ideal for moving selective acyl moieties into different phospholipid pools. Undoubtedly, the CoA-independent transacylase is most significant in its ability to transfer arachidonate from one phospholipid to another and could be a key factor in delivering arachidonate to the right phospholipid substrate for phospholipase A_2 hydrolysis so that arachidonate can be released to form eicosanoid mediators. Moreover, it appears that the CoA-independent transacylase is also directly linked to PAF formation. Thus, the CoA-independent transacylase would appear to be an excellent target for the development of an enzyme inhibitor that could intervene in the production of proinflammatory mediators such as prostaglandins, leukotrienes, HETES, lipoxins, and PAF. The myriad of acyl donor and acyl acceptor molecules that have been investigated so far indicate the selectivity of the transacylases can vary among animal species and tissues or cells from the same species. The results of the varous investigations reviewed in this article indicate the regulation of acyl transfer reactions in lipid metabolism must be very complex since all three acylation mechanisms (acyl CoA, CoA-dependent, and CoA-independent) can contribute to the fatty acid composition of phospholipids in cellular membranes.

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