## REVIEW ARTICLE

# Platelet-activating factor: the biosynthetic and catabolic enzymes

Fred SNYDER

Medical Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117, U.S.A.

## INTRODUCTION

Several reviews [1–6] have covered various aspects of recent progress in the platelet-activating factor (PAF) field, but none of these has focused solely on the specific characteristics of the individual enzymes involved in the biosynthesis and catabolism of PAF and its analogues. The need for a quick source of such information is especially timely and pertinent, since during the past few years a number of new concepts have emerged about the close relationship between PAF biosynthesis and the trafficking of arachidonate and other polyenoates among membrane phospholipids via transacylation reactions [7–10]. Moreover, Lee et al. [11] have shown that PAF can serve as an acetate donor to a variety of acceptor molecules (fatty alcohols and a number of lyso-phospholipids) in a transacetylation reaction catalysed by a CoA-independent transacetylase, and very recent studies have also demonstrated that sphingosine can be acetylated by this enzyme activity [12,13].

Considerable advances have also been made in our knowledge of the catabolism and enzymic factors responsible for regulating the metabolism of PAF. For example, the purification of PAF acetylhydrolase to near or complete homogeneity from plasma [14], erythrocytes [15] and brain [16] has greatly expanded our knowledge of this important enzyme and its role in regulating both cellular and intracellular levels of PAF.

This abridged review is intended to provide the reader with a concise summary of the functions and key properties of those enzymes known to be involved directly or indirectly in PAF metabolism and to show how these individual enzymic steps are inter-related and coordinately regulated as integrated metabolic pathways. Knowledge of the regulatory controls is limited, but these aspects, when known, are discussed for each specific enzyme. Also, in this review, a little past history has been revisited where critical enzymes in the biosynthesis of ether-linked glycerolipids were discovered in the early 1970s, since this often neglected area forms the foundation to a complete understanding of PAF metabolism. Thus, a brief description of the enzymic machinery responsible for the general intermediary metabolism of ether-linked lipids, including the formation of the *O*-alkyl and *O*-alk-1-enyl grouping, is also provided. Metabolism of PAF in intact cells and in *in vivo* systems is only discussed when it is essential to emphasize the significance of how the enzymic reactions relate to specific pathophysiological conditions.

## BIOSYNTHETIC ENZYMES

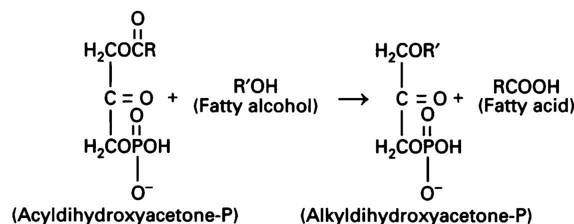
### Formation of the ether bond and related steps in the biosynthesis of ether-linked lipids

In most articles regarding PAF biosynthesis, the enzymic studies relevant to the formation of the ether bond in PAF or its plasmalogen analogue and the related metabolic pathways are usually ignored. Since such knowledge is critical to understanding the broader aspects of PAF metabolism, I have included in this section an abbreviated account of the earlier work, long before the structure of PAF was identified, that established the enzymic pathways responsible for the formation of ether linkages in glycerolipids and the corresponding intermediary reaction steps leading to more complex ether lipids (phospholipids and neutral lipids) associated with biological membranes. The reader is referred to an earlier review [17] that focuses on more detailed coverage of the properties of specific enzymes comprising the metabolic pathways for the ether-linked glycerolipids and their precursors.

#### Alkyldihydroxyacetone-P (DHAP) synthase (EC 2.5.1.26)

Almost a quarter of a century ago, the first cell-free system to synthesize *O*-alkyl moieties in glycerolipids was discovered [18]. The enzymes responsible were subsequently characterized in both cancer cells [19–22], which contain relatively high levels of ether lipids, and in normal cells [23]. The unique reaction catalysed by alkyl-DHAP synthase involves the displacement of the acyl moiety of acyl-DHAP by a long-chain fatty alcohol [24,25]; methodology for assaying this enzyme activity has recently been summarized [26,27].

A broad substrate specificity has been noted for the incorporation of the fatty alcohols into the ether linkage [28,29], and results obtained with $^{18}$O-labelled hexadecanol have shown that the oxygen of the ether linkage is derived from the alcohol [22,30]. In addition, no $^{3}$H is lost upon incorporation of [1-$^{3}$H]hexadecanol into alkyl ether lipids [31–35], which further indicates that the entire alcohol chain is transferred into the *O*-alkyl moiety. Other features of the unique reaction that forms the *O*-alkyl bond in glycerolipids are: (1) the pro R hydrogen, located at the carbon atom where the acyl moiety is attached to DHAP, exchanges with water [36–39]; (2) the configuration of the




**Scheme 1**

Abbreviations used: PAF, platelet-activating factor; DHAP, dihydroxyacetone-P; DTT, dithiothreitol; PMSF, phenylmethanesulphonyl fluoride; LCAT, lecithin–cholesterol acyltransferase; PMA, phorbol 12-myristate 13-acetate; DFP, di-isopropylfluorophosphate; c.m.c., critical micellar concentration; LDL, low-density lipoprotein; NEM, *N*-ethylmaleimide; GTP[$\gamma$S], guanosine-5′-[$\beta$-thio]triphosphate.

carbon of the DHAP moiety where the exchange occurs is preserved [40,41]; (3) the acyl moiety of acyl-DHAP is cleaved before the addition of the fatty alcohol [42,43]; and (4) both oxygens associated with the acyl moiety of acyl-DHAP remain in the fatty acid product released during the acyl exchange with the alcohol [43,44].

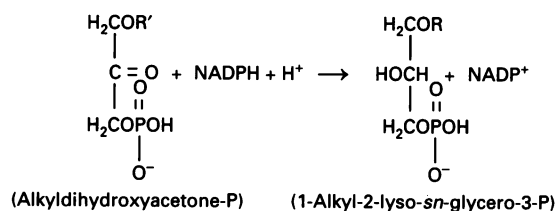
Subcellular studies by Hajra et al. [29] have indicated that both alkyl-DHAP synthase and acyl-CoA:DHAP acyltransferase activities [45–49] are highest in peroxisomes but absent in peroxisomal-deficient cells [50,51]. Nevertheless, it is noteworthy that Ehrlich ascites cells, from which alkyl-DHAP synthase has been purified 1000-fold [39,42,43], appear to be devoid of typical peroxisomal bodies. The molecular mass for the partially purified alkyl-DHAP synthase has been estimated to be approximately 300000 Da [42]. Initial velocity kinetic data obtained in experiments with this highly purified enzyme preparation, free of acylhydrolase or other competing enzymes, supports a Ping-Pong type of molecular mechanism for the alkyl-DHAP synthase-catalysed reaction [39,42,43]. The results obtained with the purified enzyme are consistent with an earlier conclusion supporting a Ping-Pong type mechanism for the reaction [52], whereby the fatty acid is first released from acyl-DHAP to give an activated enzyme–DHAP complex which can then react with a long-chain fatty alcohol to form alkyl-DHAP or with a fatty acid to reform acyl-DHAP. The fact that fatty acids compete with fatty alcohols in the formation of alkyl-DHAP lends further credence to such a mechanism; furthermore, a Schiff base is not formed as an intermediate in the alkyl-DHAP synthase catalysed reaction [53–55]. It has been proposed that a nucleophilic cofactor (possibly an amino acid functional grouping at the active site) covalently binds the DHAP portion of acyl-DHAP to form an enzyme–DHAP complex as an intermediate that reacts with the fatty alcohol to form alkyl-DHAP [39,42,43]. However, despite the strong evidence for the Ping-Pong mechanism, the possibility of a sequential type mechanism being involved in the formation of the ether bond in lipids cannot be ruled out absolutely until proof for the existence of an enzyme–DHAP complex is established.

In addition, recent reports have described a 100-fold enrichment [27] and purification to homogeneity of alkyl-DHAP synthase [56] from guinea pig liver. The completely purified enzyme exists as a single polypeptide possessing a molecular mass of 65000 Da [56]. At optimal concentrations of palmitoyl-DHAP, the $K_m$ value for hexadecanol was 72 $\mu$M and at a concentration of 0.5 mM hexadecanol the $K_m$ for palmitoyl-DHAP was 68 $\mu$M [56]; similar results [27] were obtained with the 100-fold enriched enzyme, which had $K_m$s of 45 and 40 $\mu$M for palmitoyl-DHAP and hexadecanol respectively. An analogue of acyl/alkyl-DHAP (3-bromo-2-keto-heptadecylphosphate) was found to inhibit alkyl-DHAP synthase [27]; others [57] have also described inhibitory alkyl-DHAP analogues (e.g. monopalmitoyl-1,2,3-trihydroxyeicosanephosphate).

The enzymes responsible for synthesizing acyl-DHAP and long-chain fatty alcohols, the precursors of alkyl-DHAP, have been extensively investigated, but summarizing such studies is beyond the scope of this brief review. Nevertheless, it should be noted that the formation of acyl-DHAP, the precursor of ether lipids, was first demonstrated in guinea pig liver mitochondria [58–60] and this enzyme activity has been observed in most mammalian tissues investigated. Acyl-CoA:DHAP acyltransferase has recently been partially purified (> 3200-fold) from guinea pig liver peroxisomes [61]. The other precursor, a long-chain fatty alcohol, is produced via the reduction of an acyl-CoA by an NADPH-dependent acyl-CoA reductase [17]. Chapters in two books also provide extensive coverage of the early literature on the enzymic synthesis of ether-linked lipids and their precursors in mammalian cells [17,62].

### NADPH:alkyl-DHAP oxidoreductase (EC 1.1.1.100)

The ketone grouping of DHAP, the product of the reaction catalysed by alkyl-DHAP synthase, is subsequently reduced to alkyl-*sn*-glycero-3-P by an NADPH-oxidoreductase [18,24,25,63–65].

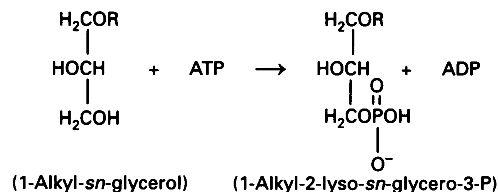



**Scheme 2**

Only the $\beta$-hydrogen of the nicotinamide ring of NADPH is utilized for the enzymic reduction of DHAP [63] and the same oxidoreductase appears to be involved in reducing both alkyl- and acyl-DHAP [65]. Although NADH at high concentrations can substitute for NADPH in the oxidoreductase-catalysed reaction, the primary requirement for NADPH becomes apparent at low concentrations of the reduced nucleotides [25,63].

### ATP:alkylglycerol phosphotransferase (EC 2.7.1.93)

Alkylglycerol-P can also be synthesized from preformed alkylglycerols by ATP:alkylglycerol phosphotransferase [66,67].

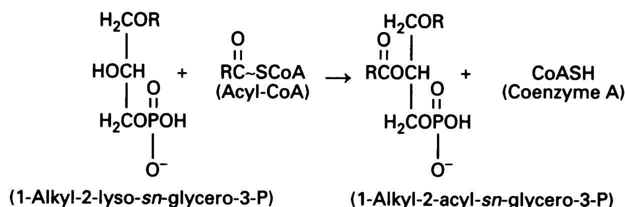



**Scheme 3**

Thus, the alkylglycerol phosphotransferase makes it possible for alkylglycerols derived from dietary intake or the catabolism of cellular ether-linked lipids to enter the biosynthetic pathways responsible for the production of PAF or structural lipid components (ether lipids with long-chain acyl moieties at the *sn*-2 position) of membrane bilayers. The alkylglycerol phosphotransferase is of microsomal origin, requires ATP and $Mg^{2+}$, exhibits a rather broad pH profile with an optimum pH at 7.1 and shows a strict stereospecificity for the *sn*-1 isomer; i.e. *sn*-glycerols possessing *O*-alkyl groups at either the *sn*-2 or *sn*-3 positions are not phosphorylated by the phosphotransferase. Also, it should be noted that alkylethyleneglycols are not substrates for this phosphotransferase and it is unknown whether the monoacylglycerol analogue can be utilized as a substrate since high lipase activities encountered in the systems studied resulted in extensive hydrolysis of the ester moiety [67]. The activity of alkylglycerol phosphotransferase can be assayed using either [$^3$H]alkylglycerol or [$\gamma^{32}$P]ATP as the radiolabelled substrate.

### Acyl-CoA:alkylglycero-P acyltransferase (EC 2.3.1.63)

1-Alkyl-*sn*-glycerol-3-P occupies a pivotal position in ether lipid biosynthesis since this metabolic intermediate is centered at a branch-point where it can be used in either the *de novo* synthesis of PAF (discussed in a later section) or the *de novo* synthesis of alkylacylglycerophosphocholines (precursors of PAF in the remodelling pathway) and alkylacylglycerophosphoethanolamines which are both membrane components. Alkylglycero-P is converted into the alkyl analogue of phosphatidic acid via an acyltransferase [25,34].



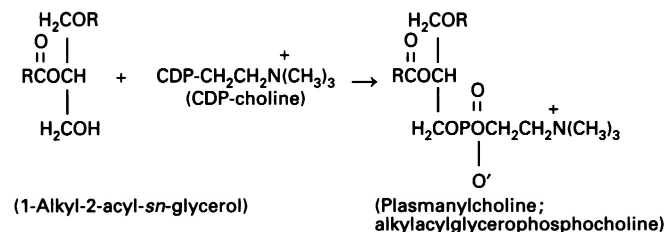

**Scheme 4**

### Alkylacylglycero-P phosphohydrolase

The phosphatidic acid analogue can be converted into 1-alkyl-2-acyl-*sn*-glycerols via a phosphohydrolase activity enhanced by $Mg^{2+}$ [68]. This phosphohydrolase has not been characterized so it is unknown whether it is the same enzyme activity as phosphatidate phosphohydrolase.

### CDP-choline:alkylacylglycerol cholinephosphotransferase (EC 2.7.8.2) or ethanolaminephosphotransferase (EC 2.7.8.1)

The alkylacylglycerol product mimics the diacylglycerols in phospholipid biosynthesis since both of them serve as acceptors for the $Mg^{2+}$-dependent transfer of the phosphobase groups of either CDP-choline or CDP-ethanolamine by a cholinephosphotransferase or an ethanolaminephosphotransferase [68].




**Scheme 5**

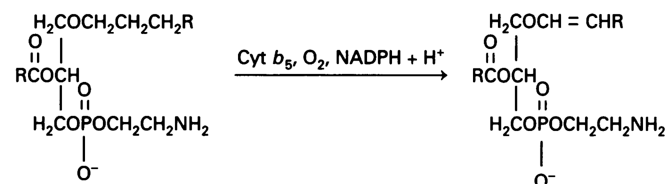
It has been reported that the diacylglycerol- and alkylacylglycerol cholinephosphotransferase activities in rat liver microsomes are the same enzyme, based on their similar pH optima, thermolabilities and inhibition by $Mn^{2+}$ and dithiothreitol (DTT) [69]. This conclusion is also supported by substrate competition experiments with microsomal fractions from the liver and brain of rats that showed that the formation of phosphatidylcholine or phosphatidylethanolamine via the cholinephosphotransferase or ethanolaminephosphotransferase respectively could be strongly inhibited by alkylacylglycerols [70].

### Acyl-CoA:alkylacylglycerol acyltransferase

The alkylacylglycerols can also be converted into alkyldiacylglycerols (an ether analogue of triacylglycerols) by an acyl-CoA acyltransferase which can also acylate 1-alk-1′-enyl-2-acyl-*sn*-glycerols to produce the plasmalogen analogue of triglycerides [71]. As will be discussed later, alkylacetylacylglycerols can be formed in a similar type of reaction catalysed by acyl-CoA:alkylacetylglycerol acyltransferase [72].

### Δ1-Alkyl desaturase (1.14.99.19)

Closely related to the alkyl ether lipids are the plasmalogens which have an *O*-alk-1-enyl ether linkage at the *sn*-1 position. This class of lipids contains a double bond (between C-1 and C-2 of the alkenyl chain) adjacent to the ether bond. In most tissues the *O*-alk-1-enyl linkage occurs primarily as an ethanolamine-containing phospholipid (plasmanylethanolamine), although in heart tissue of some species the choline plasmalogens (plasmanylcholine) predominate [73]. Enzyme studies have shown that alk-1-enyl linkages in ethanolamine plasmalogens originate from the alkyl moieties of alkylacylglycerophosphoethanolamine via a mixed-function oxidase in tumours [74], intestinal mucosa [75] and brain [76,77].




**Scheme 6**

Requirements for this reaction are molecular oxygen, NADH or NADPH, and cytochrome $b_5$ [74–78]. In this respect, the alkyl desaturase is similar to, but not identical [79] with acyl-CoA desaturase [80,81], another mixed-function oxidase; both desaturases are cyanide sensitive.

Choline plasmalogens (1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine) appear to be derived from ethanolamine plasmalogens via the combined actions of a phospholipase $A_2$, lysophospholipase D, an acyltransferase, phosphohydrolase and a cholinephosphotransferase, as well as by a direct base exchange or coupled phospholipase C–cholinephosphotransferase reactions [82]. Other findings further support the concept of ethanolamine plasmalogens being converted into choline plasmalogens via a phospholipase C [83] or other polar head group remodelling mechanism [84]. However, no evidence has ever been presented to indicate that either alkylacylglycerophosphocholine or alkyl-lysoglycerophosphocholine can serve as substrate for the Δ1-alkyl desaturase.

## The remodelling pathway

### Overall reaction sequence

As the name implies, the remodelling pathway involves a chemical modification in the structure of a pre-existing lipid molecule associated with cellular membranes. In order to produce PAF by this pathway, the *sn*-2 long-chain acyl moiety (primarily arachidonate) of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine is replaced by an acetate group. The enzymes responsible for

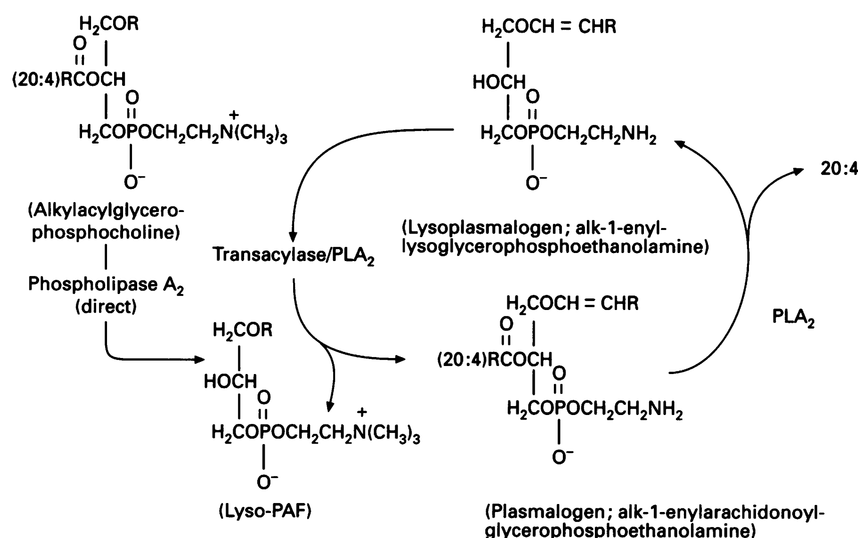
catalysing this sequence of reactions involve a deacylation and an acetylation step. The role and characteristics of each of the enzymes involved in the synthesis of PAF via the remodelling pathway are discussed in the subsequent sub-sections.

### Phospholipase A2 versus a CoA-independent transacylase/phospholipase A2 (deacylation of alkylacylglycerophosphocholine to form lyso-PAF)

Formation of the lyso-PAF intermediate from alkylacylglycerophosphocholines, the penultimate reaction step in the biosynthesis of PAF via the remodelling pathway, can occur by either a transacylation sequence involving a phospholipase $A_2$ and a CoA-independent transacylase, or directly by a selective phospholipase $A_2$ activity that utilizes alkylacylglycerophosphocholines as a substrate.

sufficient to restore the stimulation of PAF synthesis following agonist activation of the cells. Interesting results obtained wth intact human neutrophils and membrane fractions have shown that the high selectivity for arachidonate by the CoA-independent transacylase in resting cells is lost following stimulation of the cells with calcium ionophore A23187 [108].

The coupling of the CoA-independent transacylase with PAF biosynthesis has been clearly demonstrated in experiments showing that lyso-PAF, formed by the lysoplasmalogen-induced initiation of the transacylation reaction, can be readily converted into PAF in the presence of acetyl-CoA by the acetyl-CoA acetyltransferase in the remodelling pathway [9,10,105]. The lysoplasmalogen-induced transacylase activity appears to be present in a number of rat tissues tested [105], which suggests a rather broad cellular distribution of this enzyme in mammals.




**Scheme 7**

However, at the present time there is no available enzymic evidence to document the existence of a specific phospholipase $A_2$ being responsible for the direct hydrolysis of alkylacylglycerophosphocholines during cell activation. Even though a number of phospholipase $A_2$s with a preference for phospholipids containing arachidonate have been characterized [85–104], none have been directly linked to having a role in PAF biosynthesis.

On the other hand, lyso-PAF has been shown to be generated from alkylarachidonoylglycerophosphocholines via a CoA-independent transacylase found in human leukaemic (HL-60) cells [9,105], human neutrophils [10,106–109], mast cells [110] and rat tissues [105]. Several of these studies [9,10,105–108] have clearly shown a stringent requirement for arachidonate or other polyenoates at the *sn*-2 position of the alkylacylglycerophosphocholine in order to generate the lyso-PAF via the transacylation cycle. Moreover, the supplementation of arachidonic acid (10 $\mu$M) to either undifferentiated or differentiated (neutrophil form) HL-60 cells enhances both the deacylation and acyltransfer components of the CoA transacylase activity [105]. All of these findings are consistent with earlier results obtained with HL-60 cells [111] and rat peritoneal polymorphonuclear leucocytes [112] that have demonstrated that the supplementation of arachidonic acid to 20:4-depleted cells increases the precursor supply of alkylarachidonoylglycerophosphocholines to a level

These results provide compelling evidence for the significant, if not exclusive, role of the CoA-independent transacylase cycle in PAF biosynthesis. Data obtained with cultured mast cells, using defined species of lyso-PAF as the acyl acceptor substrate for the transacylase, have supported the premise that PAF is preferentially synthesized via the combined actions of the CoA-independent transacylase and the lyso-PAF acetyltransferase [110]. Furthermore, the fact that lyso-phospholipids containing acyl and alk-1-enyl (plasmalogenic) moieties at the *sn*-1 position can also be generated by the CoA-independent transacylase, suggests that the transacylation process is undoubtedly the primary mechanism for producing significant quantities of the acyl and plasmalogen analogues of PAF in many cells following agonist stimulation.

Any of the lysoglycerophospholipids containing choline or ethanolamine can serve as acceptor molecules or initiators for the arachidonate transferred from the alkylarachidonoylglycerophosphocholine pool [9,10,106,107]. In contrast, other glycerolipids with free hydroxyl groups (3-alkyl-2-lyso-*sn*-glycero-1-phosphocholine, lysophosphatidylserine, lysophosphatidylinositol, diacylglycerols, alkylglycerols and monoacylglycerols), cholesterol, phosphatidylcholine and phosphatidylethanolamine are not acyl acceptor molecules for the transacylase [9]. Also, if the *sn*-2 position does not contain a free alcohol moiety (e.g. alkylmethoxyglycerophosphocholine) the molecule will not induce the release of lyso-PAF from the alkylacylglycero-

phosphocholine precursor pool [107]. Although all of the lyso forms of the choline/ethanolamine-containing glycerophosphatides can induce the formation of lyso-PAF via a transacylation reaction, the primary source of the lysophospholipid acceptor for the transacylase appears to be ethanolamine plasmalogens, at least in human neutrophils [10,113]. When neutrophils are stimulated with calcium ionophore A23187, mass measurements showed that these cells rapidly loose arachidonate almost exclusively from ethanolamine plasmalogens and this loss is reflected by a corresponding increase in the amount of lysoplasmalogens formed [10]. Interestingly, the lysoplasmalogens that accumulate in stimulated neutrophils also act as a competing substrate for the acylation of lyso-PAF with arachidonate and, under such conditions of stimulation, the specificity for the transfer of arachidonate to lyso-PAF to form alkylarachidonoylglycerophosphocholine is lost [108].

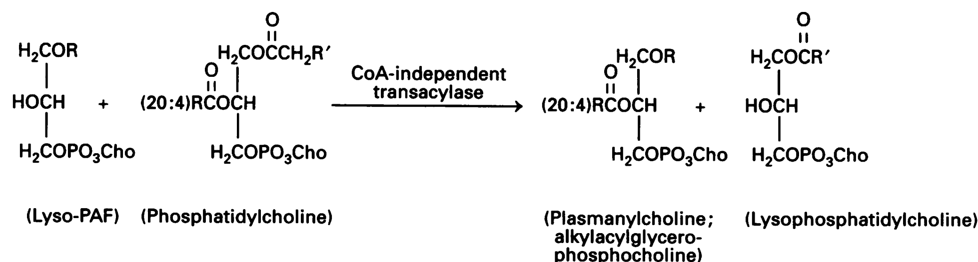
As with the other biosynthetic enzymes in PAF metabolism, the CoA-independent transacylase linked to PAF biosynthesis is associated with membranes. However, it is not known whether the hydrolytic component that removes the 20:4 of alkylarachidonoylglycerophosphocholine, and the acyl transfer activity that transfers the 20:4 to lysoplasmalogen or another acyl acceptor, reside in a single catalytic protein or in two separate entities. Such information must await the solubilization and purification of the enzymic activity(ies), an achievement that could be a formidable task since it is known that the transacylase activity is inhibited by detergents such as deoxycholate and Triton X-100 [9,107]. When it is stated that detergents inhibit the enzymic activity, the studies were done with substrates that were either below their critical micellar concentration (c.m.c.) or where ethanol or other agents were used to solubilize or disperse the lipid substrates. Initiation of the CoA-independent transacylase reaction is dependent on the availability of the lysophospholipid acceptor, and thus it would appear that the phospholipase $A_2$ responsible for generating the lyso intermediate is the rate-limiting catalytic step in the reaction sequence. This supposition has been borne out by the fact that exogenous [9,10,107] or endogenously-generated lyso-PAF [10] must be present to trigger the transacylase reaction and that inhibitors of acyl hydrolysis such as mepacrine and bromophenacyl bromide also block PAF production in intact differentiated HL-60 cells [111].

The properties of microsomal CoA-independent transacylases from a variety of cellular sources involving different types of substrates have been reviewed in depth elsewhere [7,8]. However, it is particularly relevant to at least briefly note here some of the recent findings pertaining to the characteristics of the transacylases in human neutrophils and HL-60 cells since the production of lyso-PAF in these cells has been clearly linked to the CoA-independent transacylase [9,10,107]. Experimental evidence obtained from both cell systems [9,107] have shown that the transacylase activity does not require $Ca^{2+}$ or CoA and that detergents, such as deoxycholate and Triton X-100, are strongly inhibitory. Venable et al. [107] also have demonstrated that a variety of nucleotides {ATP, GTP, guanosine-5′-[$\beta$-thio]-triphosphate (GTP[$\gamma$S]), ATP/cAMP, ATP/GTP, GTP/GTP[$\gamma$S]}, some protein kinase C activators ($Ca^{2+}$, ATP, $Mg^{2+}$, diacylglycerols) and a catalytic subunit of protein kinase A with ATP/$Mg^{2+}$ do not influence the transacylase activity in human neutrophils.

It has been reported that *N*-tosyl-L-phenylalanine chloromethyl ketone (a serine inhibitor) and diethyl pyrocarbonate (a histidine modifier) inhibits the transacylase activity in human neutrophil sonicates [107] and U937 microsomes [114]. However, conflicting results have been obtained in some studies of the properties of the CoA-independent transacylase activity among different cell types. Both phenylmethanesulphonyl fluoride (PMSF; a serine inhibitor) and *p*-bromophenacyl bromide (blocks phospholipase $A_2$ hydrolysis) have been shown to inhibit the transacylase activity (deacylation of alkylacylglycerophosphocholine and the acylation of lyso-PAF) in sonicates derived from human neutrophils [107], whereas neither compound appears to alter the transacylase activity in membranes or homogenates prepared from HL-60 cells [9,11]. Winkler et al. [114] also observed that PMSF inhibited the CoA-independent transacylase in the microsomal fraction of U937 cells but found that several phospholipase $A_2$ inhibitors (quinacrine, aristolochic acid, arachidonic acid) exerted no effect on the enzyme activity in the U937 cells. Addition of purported phospholipase $A_2$ inhibitors (aristolochic acid and scalaradial) to homogenates of human neutrophils revealed no change in the amount of radiolabelled lyso-PAF generated in the transacylase-catalysed reaction induced by unlabelled lyso-PAF [109]. The reason for the inconsistencies in these data obtained with cell-free systems from different cell types is unknown at this time. Recent findings based on the comparison of differences observed in the biochemical and pharmacological properties of the microsomal CoA-independent transacyclase from U937 cells with a low molecular mass (14000 Da) phospholipase $A_2$ from human synovial fluid and a high molecular mass (85000 Da) cytosolic phospholipase $A_2$ from U937 cells suggest these three enzymes are mechanistically and structurally different [115].

### CoA-independent transacylase (reacylation of lyso-PAF)

Many different investigations have described the acylation of lyso-PAF by the CoA-independent transacylase.



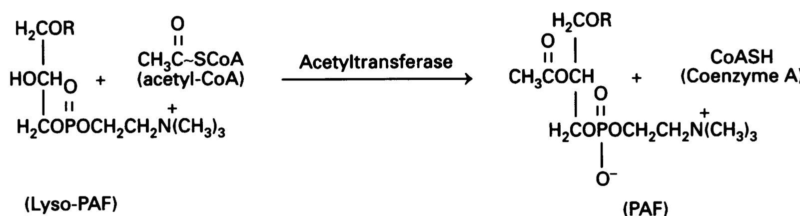

**Scheme 8**

In view of the very large scope of these studies, the reader is referred to two comprehensive reviews on the subject of transacylases [7,8] for more adequate coverage. Despite the fact that

both the deacylation of alkylacylglycerophosphocholines and the reacylation of lyso-PAF are catalysed by a CoA-independent transacylase, it has not yet been established whether a single catalytic protein is responsible for both the forward and backward reactions. However, the deacylation of alkylacylglycerophosphocholines and the acylation of lyso-PAF can be blocked in parallel by the same inhibitors and both activities respond to heat inactivation in the same manner, which suggests a single enzyme activity might possibly be involved in both reactions [107]. Such a notion is also supported by the highly selective preference of the transacylase for polyunsaturated acyl moieties in both the deacylation and reacylation reactions [105].

### Acetyl-CoA: lyso-PAF acetyltransferase (EC 2.3.1.67)

The last step in the formation of PAF in the remodelling pathway is the acetylation of lyso-PAF catalysed by acetyl-CoA: lyso-PAF acetyltransferase.




**Scheme 9**

Methods for measuring this enzyme activity, as well as relevant information about its properties, have been summarized by Lee et al. [116].

Lyso-PAF acetyltransferase occurs in the microsomal fraction of a variety of tissues [117] and blood cells [118,119] that produce PAF. A study by Mollinedo et al. [120] reported that the acetyltransferase activity is distributed in the plasma membrane, endoplasmic reticulum, tertiary granules of resting human neutrophils and in poorly defined intracellular granules following stimulation with calcium ionophore A23187. The subcellular distribution of the lyso-PAF acetyltransferase in undifferentiated HL-60 cells [121] was found to be similar to that of human neutrophils [119,120], with the primary site being the endoplasmic reticulum. In Krebs II cells, which are devoid of PAF transfer proteins, the lyso-PAF acetyltransferase activity was found to be associated with the ribosome-rich (rough) fraction of the endoplasmic reticulum [122,123].

Substrate specificity of the acetyltransferase is rather broad since 1-acyl-2-lysoglycerophosphocholine [117,124,125], 1-alk-1-enyl-2-lysoglycerophosphoethanolamine [11,126] and various other analogues, including 1-alkyl-2-lyso-*sn*-glycero-3-*N'*,*N'*-dimethylethanolamine, 1-alkyl-2-lyso-glyceromonomethylethanolamine and 1-alkyl-2-lysoglycerophosphoethanolamine [124], serve as substrates. Similarly, short-chain acyl-CoAs ($C_2$–$C_6$) can be transferred to lyso-PAF by the acetyltransferase, with propionyl-CoA having a reaction rate equivalent to acetyl-CoA [124] as the co-substrate. Thus, based collectively on these various reports, the preferred substrates for the acetyltransferase appear to be 1-alkyl-2-lysoglycerophosphocholine and acetyl-CoA or propionyl-CoA. A study of different lyso-phospholipids as potential acetate acceptors with homogenates from control and thrombin-stimulated human endothelial cells (a cell type that produces mainly the acyl analogue of PAF) indicated a specificity preference of alkyl-lysoglycerophosphocholine > acyl-lysoglycerophosphocholine ≈ alk-1-enyl-lysoglycerophosphocholine > alkyl-lysoglycerophosphoethanolamine ≈ acyl-lysoglycerophosphoethanolamine [127]. These experiments also demonstrated that acyl-lysoglycerophosphoserine, acyl-lysoglycerophosphoinositol or acyl-lysoglycero-P (lysophosphatidic acid) were not substrates for the acetyltransferase. Palmitoyl-CoA [117], oleoyl-CoA [125], arachidonoyl-CoA [128], unsaturated fatty acids [129] and 1-palmitoyl-2-lysoglycerophosphocholine [124] have been reported to be competitive inhibitors of lyso-PAF acetyltransferase. Chelating agents such as EDTA or EGTA, $Mg^{2+}$ or $Mn^{2+}$, *p*-bromophenacylbromide, *p*-chloromercuribenzoate, *N*-ethylmaleimide (NEM) and di-isopropylfluorophosphate inhibit the acetyltransferase activity [117,130,131].

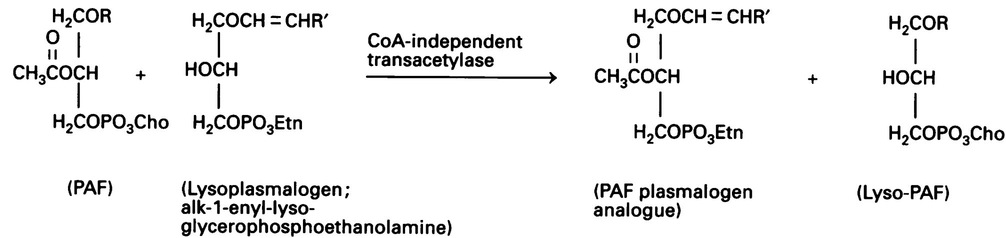
Partial purification (1500-fold) of lyso-PAF acetyltransferase from rat spleens has been reported [132], but the yield of purified enzyme was only 1.6%. The $K_m$ for acetyl-CoA with this preparation was 137 $\mu$M, which compares to a $K_m$ of 67 $\mu$M in experiments with the microsomal fraction from rat spleens [117]. Electrophoresis of the partially purified enzyme after the incorporation of labelled lyso-PAF revealed that the radioactivity was associated with a protein possessing a molecular mass of 29000 Da [132]. No subsequent studies have been described with purified preparations of the enzyme, although solubilization of the acetyltransferase from rat spleen microsomes in 25% glycerol has been claimed [131].

Lyso-PAF acetyltransferase appears to be activated and inactivated via a phosphorylation–dephosphorylation mechanism based on studies of rat spleen [133–135], human neutrophils [136], guinea pig parotid glands [137] and mouse mast cells [138] as the enzyme source. Moreover, catalytic subunits of cyclic AMP-dependent protein kinase [136,137] and calcium/calmodulin-dependent protein kinase [137] are capable of activating the acetyltransferase *in vitro*. A serine residue has been shown to be phosphorylated in studies of a partially purified preparation of acetyltransferase from rat spleen using [$\gamma$-$^{32}$P]ATP [135]. Since both phorbol myristate and 1-oleoyl-2-acetyl-*sn*-glycerol can activate the acetyltransferase, whereas sphingosine can block these effects, Leyravaud et al. [139] have concluded that protein kinase C must be involved in the phosphorylation reaction. Although the precise mechanism(s) for regulating lyso-PAF acetyltransferase is not fully understood, and may vary among cell types, it certainly appears that phosphorylation of the enzyme is required for optimal expression of the activity.

### PAF transacetylase (CoA-independent)

A recent discovery from our laboratory [11] has revealed the existence of a novel CoA-independent transacetylase that uses PAF as the acetate donor and a variety of other lyso-phospholipids (radyl-lysoglycerophosphocholines, radyl-lyso-

glycerophosphoethanolamine, acyl-lysoglycerophosphoserine, acyl-lysoglycerophosphoinositol, alkyl-lysoglycero-P and acyl-lysoglycero-P) and long-chain fatty alcohols as the acetate acceptor molecule, whereas alkylglycerols, acylglycerols and cholesterol are inactive as acetate acceptor molecules.




**Scheme 10**

Sphingosine is also known to be acetylated by the transacetylase to form *N*-acetylsphingosine [12,13]. In comparative substrate studies with acetyltransferase versus the PAF transacetylase it appears that the primary route for the biosynthesis of the plasmalogen and acyl analogues of PAF is via the CoA-independent transacetylase [11]. The importance of the transacetylase in forming the plasmalogen analogue *in vivo* is strengthened by the fact that [$^3$H]acetate from [$^3$H]PAF (but not acetate itself) can be incorporated into the plasmalogen analogue of PAF in intact HL-60 cells (differentiated into a neutrophil-type form) after being stimulated with calcium ionophore A23187.

Properties of the CoA-independent transacetylase differ from the CoA-independent transacylase in that the former exhibits a much wider range of substrate acceptor specificity and is slightly stimulated by PMSF. Also, the two activities differ in their temperature sensitivity responses: for example, at incubation temperatures of 15 and 24 °C the activity of the transacetylase is much lower (7 and 31 % respectively of control values) than the transacylase activity (44 and 81 % respectively of control values). All of these findings [11] suggest that the transacetylase and transacylase activities represent two distinctly different catalytic proteins.

It has also been reported that a purified preparation of lecithin–cholesterol acyltransferase (LCAT) from human plasma can catalyse the transfer of the acetate moiety of PAF to lysophosphatidylcholine [140]. The LCAT and transacetylase activities appear to be two separate enzymes since the transacetylase has an intracellular location, a lower molecular mass and does not transfer either long-chain acyl or acetyl moieties from phosphatidylcholine to cholesterol. The transacetylase also is not inhibited by PMSF and exhibits a broad specificity toward acetyl acceptor lipids [11].

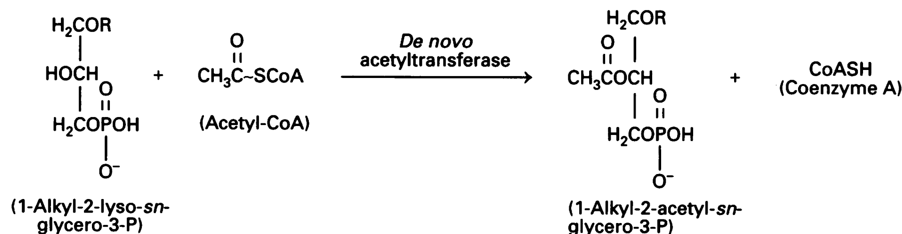
## The *de novo* pathway

### Overall reaction sequence

1-Alkyl-2-lyso-*sn*-glycero-3-P, an important metabolic ‘lyso’ intermediate in the biosynthesis of ether-linked phospholipids that possess a long-chain acyl moiety at the *sn*-2 position, represents the branch-point for the initial enzymic step in the *de novo* synthesis of PAF. The sequence of reactions in the *de novo* pathway consists of acetylation, dephosphorylation and choline-P transfer steps catalysed respectively by an acetyltransferase [141], a phosphohydrolase [142] and a cholinephosphotransferase [143,144]. Each of these enzymes has different properties than those involved in the PAF remodelling pathway or other established pathways of phospholipid biosynthesis. All three enzymes are associated with membranes and none have yet been purified. The role and properties of each of these enzymes in the *de novo* synthesis of PAF are described in the remaining portions of this section.

### Acetyl-CoA:alkyl-lysoglycero-P acetyltransferase (EC 2.3.1.105)

The microsomal acetyltransferase in the *de novo* pathway, like its counterpart in the remodelling route, utilizes acetyl-CoA as the acetate donor. However, the two activities exhibit discrete differences in their preference for lyso-phospholipid acceptors and acyl donors that serve as co-substrates in the reaction, as well as in other properties such as their heat sensitivity [141].




**Scheme 11**

It is necessary to measure alkyl-lysoglycero-P acetyltransferase in the presence of vanadate and fluoride ions and at lower temperatures (e.g. 23 °C) in order to minimize the phosphohydrolase activity that is also present in microsomal preparations. In the absence of the phosphohydrolase inhibitors, alkyl-acetylglycerol is the principal product. The pH optimum for the acetyltransferase is rather broad (pH 8.0–8.8), with a maximum activity observed at pH 8.4 in microsomes from rat spleen as the enzyme source [141]. In these studies, the apparent $K_m$ for acetyl-CoA was 226 $\mu$M and the optimal concentrations for alkyl-

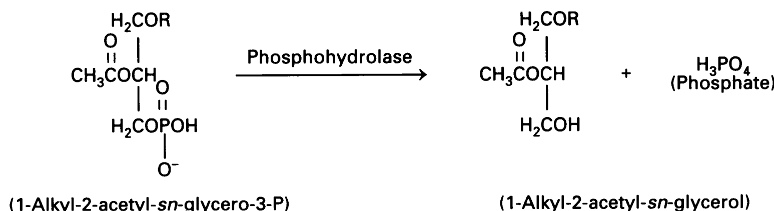
lysoglycero-P (the co-substrate) ranged between 16 and 25 $\mu$M. It appears that the *de novo* acetyltransferase is ubiquitously distributed in mammalian cells since significant activities are present in a variety of rat tissues [141].

Substrate specificity experiments have revealed that the acyl analogue (1-oleoyl-2-lyso-*sn*-glycero-3-P) can also be acetylated by the acetyltransferase, albeit at a considerably lower rate than the 16:0 or 18:0 alkyl-lysoglycero-P. Acyl-CoAs possessing from $C_2$ to $C_6$ chains are all excellent *sn*-2 acyl donors in the acetyltransferase catalysed reaction. In fact, even long-chain acyl-CoAs participate as substrates for the acetyltransferase but the activity under these circumstances was 27% less for arachidonoyl-CoA and 80% less for linoleoyl-CoA than the rates obtained for acyl-CoAs having the shorter acyl chains. In contrast, the acetyltransferase in the remodelling pathway prefers either acetyl-CoA or propionyl-CoA and is inactive with acyl-CoAs with carbon chains beyond the hexanoyl (6:0) species [124].

The fact that the acetyltransferase activity is lower than either the phosphohydrolase or cholinephosphotransferase activities in the *de novo* route for all rat tissues examined [145] implies that this acetylation reaction is a rate-limiting step in the *de novo* synthesis of PAF. Evidence has also been presented to suggest that the acetyltransferase in the *de novo* route is regulated by phosphorylation–dephosphorylation [146,147]. The changes in the activity of the acetylglycero-P acetyltransferase induced by phorbol 12-myristate 13-acetate (PMA) indicates that phosphorylation of the enzyme is probably catalysed by protein kinase C [146]. All of these findings indicate that alkyl-lysoglycero-P acetyltransferase is an important regulatory enzyme in the *de novo* route. However, as discussed later, it is noteworthy that cytidylyltransferase (produces CDP-choline) and that the DTT-insensitive cholinephosphotransferase can play an important role in regulating the *de novo* synthesis of PAF.

### Alkylacetylglycero-P phosphohydrolase (EC 3.1.3.59)

Alkylacetylglycero-P phosphohydrolase catalyses the removal of the phosphate moiety from alkylacetylglycero-P [142,148] in an intermediary reaction that bridges the initial and final steps of the *de novo* pathway.




**Scheme 12**

Thus, this enzyme has a role similar to phosphatidate phosphohydrolase in the biosynthesis of phosphatidylcholine. However, there are a number of differences in the properties of the alkylacetylglycero-P and phosphatidate phosphohydrolases to suggest that these activities represent two separate catalytic proteins. For example, when assays of alkylacetylglycerol-P phosphohydrolase and phosphatidate phosphohydrolase are done under the same conditions (i.e. substrates solubilized in ethanol and identical microsomal preparations from rat spleen used for the assays), the optimal pHs are 7.4 and 6.2 for alkylacetylglycero-P phosphohydrolase and phosphatidate phosphohydrolase respectively. When using the optimal assay conditions for alkylacetylglycero-P phosphohydrolase, the phosphatidate phosphohydrolase with dioleoylglycero-P as the substrate had 80% less activity than when alkylacetylglycerol was the substrate. The two types of phosphohydrolase activities also exhibit differences in their response to temperature and detergents. The activity of phosphatidate phosphohydrolase increases over a temperature range up to 60 °C, whereas the activity of alkylacetylglycero-P phosphohydrolase already begins to decrease above 37 °C. Furthermore, the latter activity is severely inhibited ($\approx 60\%$) by 12 mM deoxycholate, a concentration that stimulates the phosphatidase activity. Although both phosphohydrolases are inhibited by $Ca^{2+}$ (0.5–10 mM) and $Mg^{2+}$ (> 0.5 mM), the activity of phosphatidate phosphohydrolase is actually stimulated at lower levels of $Mg^{2+}$ (0.1 mM). Even at the higher concentrations of $Mg^{2+}$ (5 mM), phosphatidate phosphohydrolase is only inhibited to a level of 56% of the control value compared with an inhibition of 26% of the control value for alkylacetylglycero-P phosphohydrolase. A paper summarizing the specific assay method for this enzyme activity has been published [148].

Alkylacetylglycero-P phosphohydrolase is widely distributed among various rat tissues [142], which further suggests the importance of the *de novo* pathway in producing the endogenous levels of PAF found in mammalian cells and blood. The highest phosphohydrolase activities in microsomal preparations isolated from various rat tissues were from kidney > brain > spleen > lung > liver.

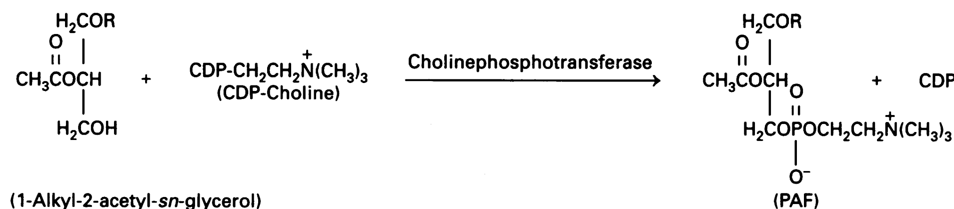
The alkylacetylglycerol intermediate occupies a particularly important position in the *de novo* pathway. This metabolite is not only the immediate precursor of PAF in this route, but the alkylacetylglycerols are also capable of triggering the differentiation of HL-60 cells into cells resembling mononuclear phagocytes [149] and attenuating the stimulation of protein kinase C by diacylglycerols [150].

### DTT-insensitive CDP-choline:alkylacetylglycerol cholinephosphotransferase (EC 2.7.8.16)

The final step in the *de novo* pathway of PAF biosynthesis is catalysed by a DTT-insensitive cholinephosphotransferase [143,144,151] that transfers the phosphocholine moiety from CDP-choline to 1-alkyl-2-acetyl-*sn*-glycerol.

A synopsis of the assay system needed to measure the cholinephosphotransferase in the *de novo* synthesis of PAF is available [151].

The availability of CDP-choline, which is formed by cytidylyltransferase, can be rate-limiting in the production of PAF by this pathway. Therefore, any factor or condition that activates cytidylyltransferase, such as fatty acids [152,153], or elevates the




**Scheme 13**

intracellular levels of CDP-choline [154] can enhance the biosynthesis of PAF via the *de novo* pathway (see next subsection).

'DTT-insensitive' is not entirely accurate in describing the *de novo* cholinephosphotransferase since DTT actually stimulates this cholinephosphotransferase to a small extent. Nevertheless, the term 'DTT-insensitive' is helpful to distinguish the *de novo* cholinephosphotransferase from the DTT-sensitive cholinephosphotransferase that is responsible for catalysing the biosynthesis of the commonly occurring phosphatidylcholine or plasmanylcholine since the latter activity is strongly inhibited by DTT, reaching > 95 % inhibition at a concentration of 10 mM DTT. Other sulphydryl reagents (2-mercaptoethanol, reduced glutathione and cysteine) have no effect on the *de novo* enzyme and also exert little influence on the DTT-sensitive cholinephosphotransferase that forms phosphatidylcholine.

The differences in DTT sensitivity have suggested that the cholinephosphotransferase activities involved in the biosynthesis of PAF and phosphatidylcholine reside in separate proteins. This supposition is further strengthened by other intrinsic differences in the properties of the two cholinephosphotransferase activities [144]. For example, the optimal pH for the *de novo* cholinephosphotransferase is 8.0 versus 8.0–9.0 for the cholinephosphotransferase in phosphatidylcholine biosynthesis. Also, the former activity is strongly inhibited by deoxycholate, whereas the latter activity is stimulated at low concentrations (0.05–0.50 mM) of this detergent. In addition, the DTT-insensitive cholinephosphotransferase is considerably more stable to elevated temperatures than the DTT-sensitive enzyme. Another notable difference between the two cholinephosphotransferase activities in microsomes isolated from rat kidney is in their sensitivity to ethanol, the solvent required for solubilizing the diradylglycerol substrates. The DTT-insensitive cholinephosphotransferase activity is significantly reduced at concentrations of ethanol above 2–3 % in contrast to the DTT-sensitive cholinephosphotransferase activity which remains relatively constant over a concentration range of ethanol from 2.5 to 5 %. One exception with regard to ethanol sensitivity has been reported for rat brain microsomes where concentrations of 2–5 % ethanol were found to inhibit the DTT-insensitive cholinephosphotransferase [155].

The cholinephosphotransferases involved in both PAF and phosphatidylcholine biosynthesis have similar requirements for $Mg^{2+}$ (10–20 mM) and although $Mn^{2+}$ can also substitute for $Mg^{2+}$, it is less effective in sustaining as high an enzyme activity at equimolar concentrations. In contrast, $Ca^{2+}$ (> 10 $\mu$M) inhibits the cholinephosphotransferase activities of both pathways [144].

As with the other enzymes of the *de novo* pathway, inflammatory stimuli have no effect on the DTT-insensitive cholinephosphotransferase. On the other hand, physiological factors such as acetylcholine and dopamine are capable of activating the *de novo* cholinephosphotransferase [156,157]. PMA is also capable of stimulating the DTT-insensitive cholinephosphotransferase activity in human neutrophils [158] and endothelial cells [146]. A similar activation of this enzyme has been seen when human neutrophils are treated with increasing concentrations of 1-oleoyl-2-acetylglycerol [159], which suggests the possibility for a role of protein kinase C in regulating the *de novo* pathway of PAF biosynthesis. During the development of experimental necrosis of the renal medulla induced by 2-bromoethylamine hydrobromide, the DTT-insensitive cholinephosphotransferase is selectively inhibited without any change in the activity of other enzymes in the *de novo* reaction sequence [160]. The fact that the activity of the DTT-insensitive cholinephosphotransferase can be both negatively and positively influenced suggests that this final step in the *de novo* synthesis of PAF could also have an important role in the regulation of endogenous PAF levels.

### CTP: phosphocholine cytidylyltransferase (EC 2.7.7.15)

It is well established that one of the rate-limiting steps in the biosynthesis of phosphatidylcholine is the formation of CDP-choline (the substrate for cholinephosphotransferase) in a reaction catalysed by cytidylyltransferase [161–163].

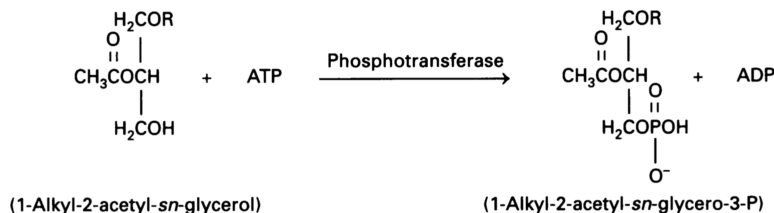
**Scheme 14**

Therefore, it is not surprising that the cytidylyltransferase reaction is also rate-limiting in the *de novo* synthesis of PAF [152–154]. These investigations documented that PAF synthesis can be significantly stimulated in Ehrlich ascites cells [152], HL-60 cells [153] and rabbit platelets [154] after treatment with oleic acid. The latter is known to cause the activation of cytidylyltransferase in a variety of cells [161–163], including those where stimulation of PAF biosynthesis also occurs [152–154]. In addition, it has been shown that by increasing the level of CDP-

choline in saponin-permeabilized HL-60 cells, the conversion of alkylacetylglycerols into PAF is greatly increased [154]. All of these findings clearly demonstrate that cytidylyltransferase is a rate-controlling enzyme in the production of PAF via the *de novo* pathway.

### ATP:alkylacetylglycerol phosphotransferase

Only a brief report [164] has described some of the characteristics of an ATP-dependent phosphotransferase in microsomal preparations from rabbit platelets that catalyses the phosphorylation of alkylacetylglycerols.

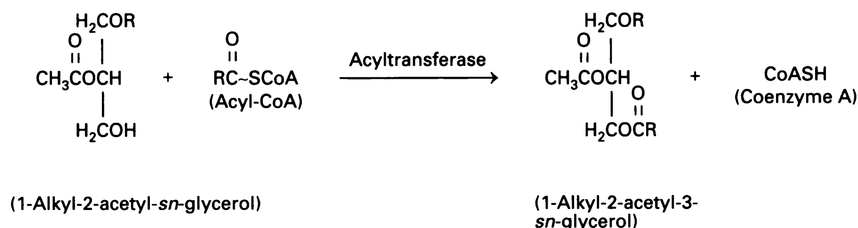



**Scheme 15**

Maximum reaction rates occur when the lipid substrate is dissolved in 1 % ethanol and the microsomes are incubated with 2–10 mM ATP-$Mg^{2+}$ at pH 7.2. Since similar results were obtained when diacylglycerols and oleoylacetylglycerols were tested as substrates, it would appear that a single phosphotransferase probably utilizes both the ether- and ester-linked substrates.

## Biosynthesis of alkylacetylacylglycerols by an acyl-CoA:alkylacetylglycerol acyltransferase (EC 2.3.1.125)

Alkylacetylglycerols, formed via the *de novo* pathway or by phospholipase C hydrolysis of PAF, can be acylated by an acyltransferase to form an acetylated *O*-alkyl analogue of triglycerides [72].




**Scheme 16**

The acyltransferase activity detected in a microsomal fraction isolated from HL-60 cells has a pH optimum of 6.5 and does not require divalent cations. The enzyme prefers linoleoyl-CoA over a variety of other acyl-CoA substrates (8:0–20:4) tested. $K_m$ and $V_{max.}$ values were 8.5 $\mu$M and 1.7 nmol·$min^{-1}$·$mg^{-1}$ of protein respectively when 1-hexadecyl-2-acetyl-*sn*-glycerol was the substrate for the acyltransferase from HL-60 cells. The acyltransferase that utilizes the alkylacetylglycerols as substrates appears to be different from the acyltransferase that uses diacylglycerols, since the former has a very sharp pH optimum at 6.8 whereas the latter has a broad pH optimum reaching a maximum at 6.1; also the diacylglycerol acyltransferase is stimulated by $Mg^{2+}$ and exhibits different substrate specificities and a greater degree of temperature sensitivity than the alkylacetylglycerol acyltransferase. Moreover, substrate competition experiments with 1-hexadecyl-2-oleoyl-*sn*-glycerol and 1-oleoyl-2-acetyl-*sn*-glycerol indicated that these lipids behave as competitive and mixed types of inhibitors respectively in the synthesis of 1-hexadecyl-2-acetyl-3-acylglycerol. All of these results suggest that the alkylacetylglycerol acyltransferase activity is uniquely different from other diradylglycerol acyltransferases.

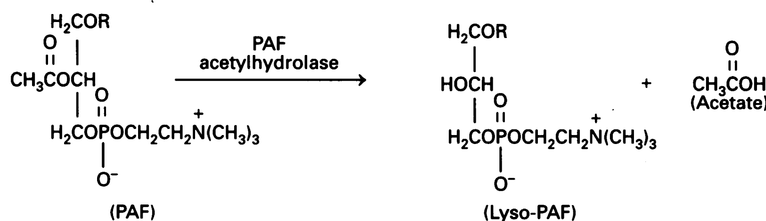
Although the function of the alkylacetylglycerol acyltransferase is not established, the fact that its activity is expressed in intact cells [72] indicates that the neutral acetylated ether lipid product might be of physiological significance. It has been proposed that the acetylated ether analogue of triglycerides might represent a storage form of acetylated lipids that could, upon the action of a lipase, become available as a precursor (alkylacetylglycerols) for PAF biosynthesis.

## CATABOLIC ENZYMES

### PAF acetylhydrolase (EC 3.1.1.48)

Deacetylation of PAF to lyso-PAF is achieved by a $Ca^{2+}$-independent PAF acetylhydrolase, an enzyme that is distributed ubiquitously in mammalian tissues and blood [165,166] and also occurs in lower animal species [167–170].

Initial studies characterized some of the properties of both the extracellular [171–174] and intracellular [165] forms of PAF acetylhydrolase; the primary difference between the extracellular and intracellular acetylhydrolases in these crude preparations are that the latter form is resistant to proteolysis [166,174]. The extracellular acetylhydrolase is loosely bound to lipoproteins in blood [14,175,176], whereas the intracellular acetylhydrolase




**Scheme 17**

**Table 1 Characteristic properties of several purified acetylhydrolases**

| | Enzyme source | | |
|---|---|---|---|
| | Human plasma [14] | Human erythrocyte [15] | Bovine brain [16] |
| Fold purification | 25000 | 15600 | 2000 |
| Molecular mass (Da) | 43000 | 25000 | 100000 |
| Specific activity ($\mu$mol · min$^{-1}$ · mg$^{-1}$ of protein) | 9.47 | 0.80 | 1.45 |
| $K_m$ | 13.7 | 12.5 | – |

has been found in both the cytosolic and membrane fractions of rat liver [165], Krebs II ascites cells [122] and HL-60 cells [177].

Both the intracellular and extracellular forms of the PAF acetylhydrolase exhibit similar substrate specificities [14–16,178]. In addition to hydrolysing the acetyl group of PAF, acetylhydrolase is also able to utilize other phospholipids with short-chain *sn*-2 acyl groups as substrates, including oxidized phospholipid fragments (see below). Specific techniques for assaying PAF acetylhydrolase have been summarized [179,180].

The acetylhydrolase activities associated with human plasma [14], human erythrocytes [15] and bovine brain [16] have been purified and some of their properties are summarized in Table 1. Differences in the behaviour of these purified enzymes towards various reagents have been reported. Acetylhydrolase isolated from human erythrocytes requires the addition of sulphydryl agents for optimal activity and is inhibited by 5,5′-dithiobis(2-nitrobenzoic acid), NaF, diethyl pyrocarbonate and *p*-bromophenacyl bromide (which derivatizes histidine residues) and proteases [15,166], whereas none of these treatments affect the purified enzyme activity isolated from human plasma. Heavy metals such as cadmium, lead and copper have also been shown to inhibit the erythrocyte acetylhydrolase, presumably through their interaction with sulphhydryl groups [166]. Di-isopropyl fluorophosphate (DFP) and *p*-nitroguanidinobenzoate (which derivatizes serine residues) strongly inhibit the acetylhydrolase from erythrocytes but do so to a much lesser extent with the enzyme from human plasma [166]. The acetylhydrolase from bovine brain is also inhibited by *p*-bromophenacylbromide and DFP, which further suggests the existence of essential histidine and serine residues at the active site [16]. However, like the plasma enzyme, there was no indication that free sulphydryl groupings are essential for the expression of maximum activity by the bovine enzyme, since iodoacetamide (an SH group inhibitor) had no effect on the enzyme activity [16]. At concentrations of PAF greater than the c.m.c. of approximately 2.5–3.0 $\mu$M [165], detergents inhibit the acetylhydrolase activity [14,15,166]. Furthermore, based on dilution kinetics, it appears that the hydrolysis of PAF by acetylhydrolase occurs at lipid interfaces, where it serves as a scavenger enzyme for both PAF and oxidized phospholipid fragments [15].

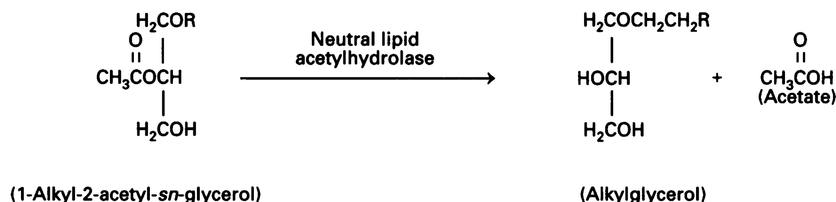
The active form ($\approx$ 60–70 %) of the acetylhydrolase in plasma is associated with the low-density lipoprotein (LDL) fraction, whereas the remaining activity associated with the high-density lipoproteins is much less efficient in hydrolysing the acetate moiety of PAF [14,175,176]. Although the acetylhydrolase can be transferred in either direction between both of these lipoprotein particles, the much higher activity in the LDL fraction appears to be due to how the substrate itself (i.e. PAF) distributes preferentially into the LDL particles [175]. Other experiments where the acetylhydrolase activity in either lipoprotein fraction was first inactivated and then reconstituted with the active enzyme from the opposite particle also demonstrated that the acetylhydrolase activity was most pronounced in the LDL particles [176].

PAF acetylhydrolase seems also to be of considerable relevance to diseases other than inflammatory disorders. For example, oxidized phospholipid fragments, possessing short-chain acyl moieties at the *sn*-2 position, in addition to PAF, can serve as substrates for PAF acetylhydrolase [15,181–183]. These findings are especially noteworthy since oxidized phospholipid fragments are thought to play a significant role in the transformation of macrophages to foam cells during the development of atherosclerotic plaques in the vascular compartment [184,185].

Various cells could be a contributing source of the PAF acetylhydrolase in plasma since it has been shown that the acetylhydrolase can be secreted by platelets [186,187], hepatocytes [188], HepG2 cells [188,189], macrophages [179,190–192] and HL-60 cells following their differentiation into macrophage-like [192] or neutrophil-like [177] forms. Moreover, factors such as hormones [188,192–194], cigarette smoke [195], a bacterial endotoxin, lipopolysaccharide, tumour necrosis factor-$\alpha$ and interleukins 1$\alpha$ and 1$\beta$ [196] can markedly influence the secretion of PAF acetylhydrolase. It is also known that many diseases and other conditions are capable of altering the levels of PAF acetylhydrolase in plasma (see review [6]).

### Alkylacetylglycerol acetylhydrolase

Another type of $Ca^{2+}$-independent acetylhydrolase hydrolyses the acetate of alkylacetylglycerols, the immediate precursor of PAF in the *de novo* biosynthetic pathway [197].

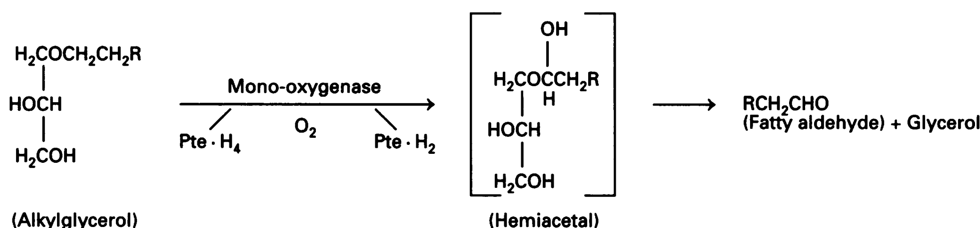



**Scheme 18**

This enzyme activity has been partially characterized in Ehrlich ascites cells where the bulk of the activity (> 90% of the total) resides with membrane fractions, which differs from PAF acetylhydrolase where the major portion of activity is in the cytosolic fraction. Kinetic studies done at physiological pH (7.5), in order to avoid isomerization of the acetyl group, gave an apparent $K_m$ and $V_{max.}$ of 45 $\mu$M and 179 $nmol \cdot min^{-1} \cdot mg^{-1}$ of protein respectively. Calcium, magnesium, EDTA, nitrophenylacetate (an esterase substrate), *p*-chloromercuribenzoate or NEM did not affect the enzyme activity, whereas NaF was highly inhibitory. Both the subcellular distribution and the NaF results indicate that the alkylacetylglycerol acetylhydrolase activity is different from the PAF acetylhydrolase. Data from these experiments also showed that the alkylacetylglycerol acetylhydrolase is not the same as diacylglycerol lipase, monoacylglycerol lipase or a non-specific esterase [197].

### Alkylglycerol mono-oxygenase (EC 1.14.16.5)

Cleavage of the *O*-alkyl linkage in glycerolipids is catalysed by a mono-oxygenase that requires tetrahydropteridine ($Pte \cdot H_4$); this enzyme is often referred to as the alkyl cleavage enzyme. The proposed transient intermediate in this reaction is an unstable hemiacetal that spontaneoulsy breaks down to produce a long-chain fatty aldehyde as the lipid product. The latter can either be reduced to a fatty alcohol or oxidized to a fatty acid by an oxidoreductase [17].




**Scheme 19**

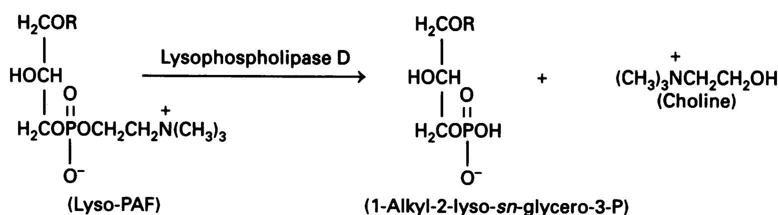
The mechanism of oxidative attack in forming the hemiacetal, originally proposed by Tietz et al. [198], is based on the hydroxylation step for phenylalanine as described by Kaufman [199].

In addition to the oxygen requirement [198], the cleavage enzyme exhibits maximum activity in the presence of ammonium ions, sulphydryl groups and a heat-labile, non-dialysable component from the soluble fraction of cells; the pH optimum of this enzyme is 9.0 [200]. Only a single investigation has focused on the involvement of the alkyl mono-oxygenase in PAF metabolism [201] and these results documented that PAF is not a substrate; however, the data did demonstrate that the *O*-alkyl moiety of lyso-PAF could be cleaved by the enzyme. These findings are in agreement with earlier experiments that demonstrated that glycerophospholipids (e.g. octadecylglycero-P) must contain at least one free hydroxyl moiety in order to be a substrate for the alkyl cleavage enzyme [202]. Thus, the alkyl mono-oxygenase could act in concert with acetylhydrolase in the degradation of PAF to a lipid product (e.g. fatty aldehydes) not containing glycerol. However, most cells [203], other than liver and intestines [204,205], possess very little alkyl cleavage enzyme activity. Thus, this mono-oxygenase would not appear to be a significant factor in the catabolism of PAF.

### Lysophospholipase D (EC 3.1.4.39)

Lysophospholipase D is a microsomal enzyme that only attacks ether-linked lysoglycerophosphatides containing either choline or ethanolamine [206–209]. PAF is not a substrate for lysophospholipase D provided that inhibitors (*p*-bromophenacyl bromide, di-isopropylfluorophosphate) are present to block the initial hydrolysis of the *sn*-2 acetate moiety by PAF acetylhydrolase [208]. The products of the lysophospholipase D-catalysed reaction with lyso-PAF as the substrate are 1-alkyl-2-lyso-*sn*-glycero-P and choline.

Lysophospholipase D from rat tissues requires $Mg^{2+}$ ions [206,207], whereas the same activity from rabbit kidneys requires $Ca^{2+}$ [209]. The enzyme in rat tissue microsomes is not stimulated by $Ca^{2+}$ and, in fact, at concentrations of $Ca^{2+}$ ranging from 1 mM to 10 mM, the activity of the $Mg^{2+}$-activated enzyme is inhibited [207]. In contrast, the renal lysophospholipase D from rabbits is not affected by $Mg^{2+}$ ions [209]. The difference in

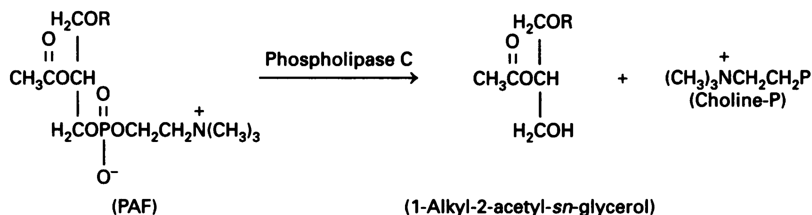



**Scheme 20**

enzymic properties between animal species was also observed in the pH optima for lysophospholipase D (7.0–7.6 rat liver; 8.0–8.6 rabbit kidney). Results obtained in studies of lysophospholipase D in rat liver microsomes [207] demonstrated that the activity could be inhibited by sulphydryl reagents [*p*-hydroxy-mercuribenzoate, 5,5′-dithiobis(2-nitrobenzoic acid), NEM, iodoacetamide], detergents (Triton X-100, deoxycholate) and low-temperature extraction with organic solvents (butanol, acetone). Another distinction among animal species is that SH group reactive agents have no influence on lysophospholipase D activity in rabbit kidney microsomes [209], which is the opposite to the data obtained with the rat liver enzyme. Resistance of the enzyme activity to trypsin indicates that the lysophospholipase D in rat liver is tightly bound or internalized within microsomal vesicles [207].

### Phospholipase C (EC 3.1.4.3)

A phospholipase C activity in a light mitochondrial fraction of rabbit liver has been reported to catalyse the hydrolysis of the phosphocholine moiety of PAF to produce alkylacetylglycerols [210].



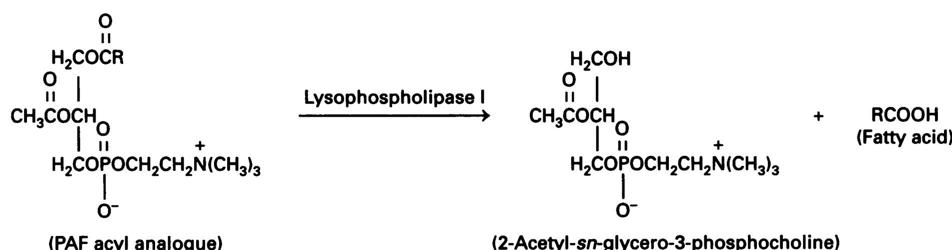

**Scheme 21**

The enzyme, which appears to be of lysosomal origin, was purified 600–700-fold after being solubilized from the mitochondrial fraction using 2% Triton X-100. Two forms of this phospholipase C activity, possessing molecular masses of 33000 and 75000 Da, had $K_m$ values of 55.6 and 45.5 $\mu$M and pH optima of 8.2 and 8.5 respectively, were identified in this investigation. A high degree of specificity of the enzyme activity was observed for PAF as a substrate, although some activity toward other phospholipids was also described. EDTA inhibited the enzyme activity and this inactivation could be restored by $Ca^{2+}$ ions. The presence of SH groups at the active site of the enzyme was suggested by the inhibitory action of *p*-chloromercuribenzoate on the phospholipase C activity.

The possibility of a phospholipase C being involved in the metabolism of PAF by intact cells has also been described in studies of PAF metabolism carried out in cultured adult rat hepatocytes [211]. However, the overall role of phospholipase C in the inactivation of PAF does not appear to be too important, since many other investigations of PAF metabolism or PAF-related enzyme systems have never provided any evidence to support the involvement of a phospholipase C activity in PAF catabolism. Moreover, the high specific activities of PAF acetyl-hydrolase found in almost all cells and tissues examined so far would seem to preclude the significance of a phospholipase C activity in the catabolism of PAF, even when present.

### Lysophospholipases I and II

Lysophospholipases I and II appear to be involved in the catabolism of the acyl PAF and the acyl analogue of lyso-PAF since these enzymic activities are able to catalyse the hydrolysis of the acyl moiety at the *sn*-1 position and in some instances the acetate at the *sn*-2 position.




**Scheme 22**

It has been suggested that lysophospholipases could determine the relative proportion of the acyl versus ether-linked forms of PAF present in cells [212–216]. Cell-free systems from mouse mast cells [215] and human neutrophils [214] indicate that lysophospholipase and acetylhydrolase activities participate in the metabolic breakdown of the acyl analogue of PAF. Moreover, the purified lysophospholipases I and II from bovine liver are capable of hydrolysing the acyl moiety of acyl-PAF. In addition, the lysophospholipase II can also hydrolyse the acetyl moiety of PAF [213]. However, the purified lysophospholipases from rat kidney and human and rat platelets are devoid of PAF acetylhydrolase activity [213]. Despite these reports, evidence is still

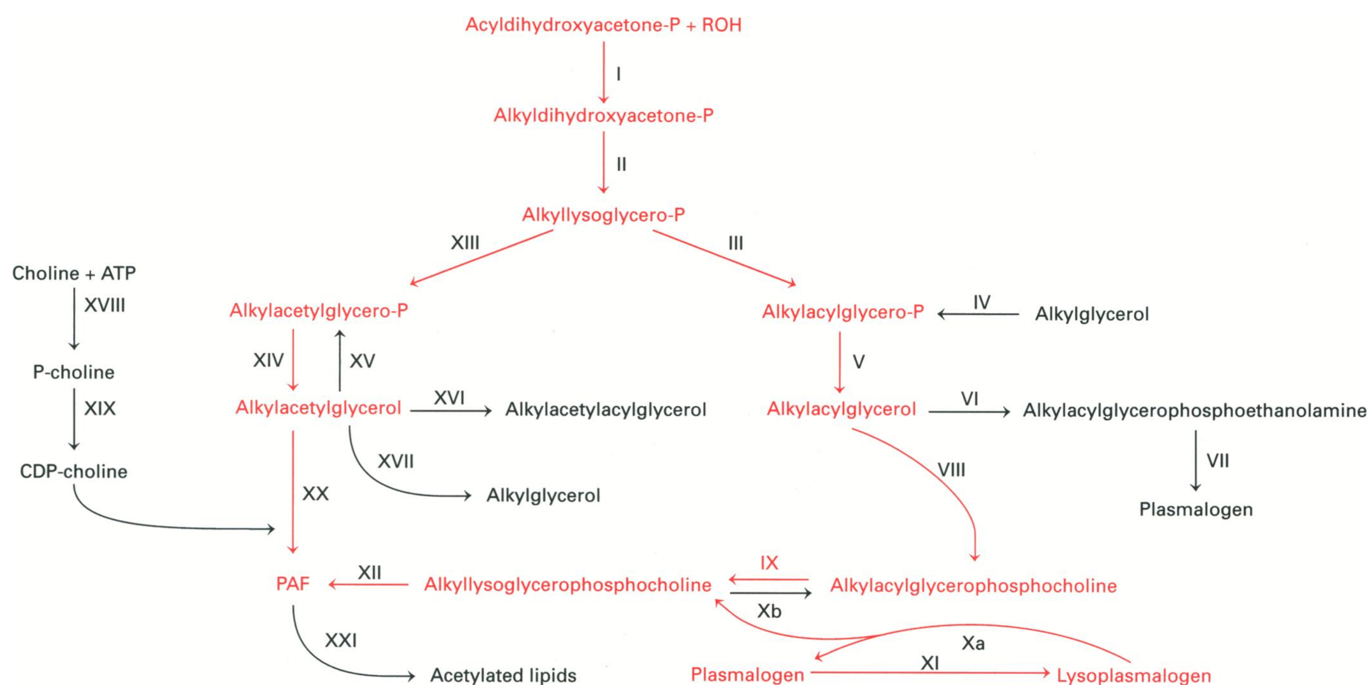



**Figure 1 Metabolic pathways for the biosynthesis of PAF and related ether-linked glycerolipids**

The Roman numerals designate the following enzymes: I, alkyl-DHAP synthase; II, NADPH:alkyl-DHAP oxidoreductase; III, acyl-CoA:alkylglycero-P acyltransferase; IV, ATP:alkylglycerol phosphotransferase; V, alkylacylglycero-P phosphohydrolase; VI, CDP-ethanolamine:alkylacylglycerol ethanolaminephosphotransferase; VII, Δ1-alkyl desaturase; VIII, CDP-choline:alkylacylglycerol cholinephosphotransferase; IX, phospholipase A2 (direct deacylation of alkylacylglycerophosphocholine substrate); Xa, CoA-independent transacylase (deacylation of alkylacylglycerophosphocholine via transacylation of lysoplasmalogen); Xb, CoA-independent transacylase (reacylation of lyso-PAF); XI, phospholipase A2 (plasmalogen substrate); XII, acetyl-CoA:lyso-PAF acetyltransferase; XIII, acetyl-CoA:alkyllysoglycero-P acetyltransferase; XIV, alkylacetylglycero-P phosphohydrolase; XV, ATP:alkylacetylglycerol phosphotransferase; XVI, acyl-CoA:alkylacetylglycerol acyltransferase; XVII, alkylacetylglycerol acetylhydrolase; XVIII, ATP:cholinephosphotransferase; XIX, CTP:phosphocholine cytidylyltransferase; XX, DTT-insensitive CDP-choline:alkylacetylglycerol cholinephosphotransferase; and XXI, PAF transacetylase (CoA-independent). Reactions shown in red designate the main enzymic pathways responsible for the biosynthesis of PAF and its precursors.

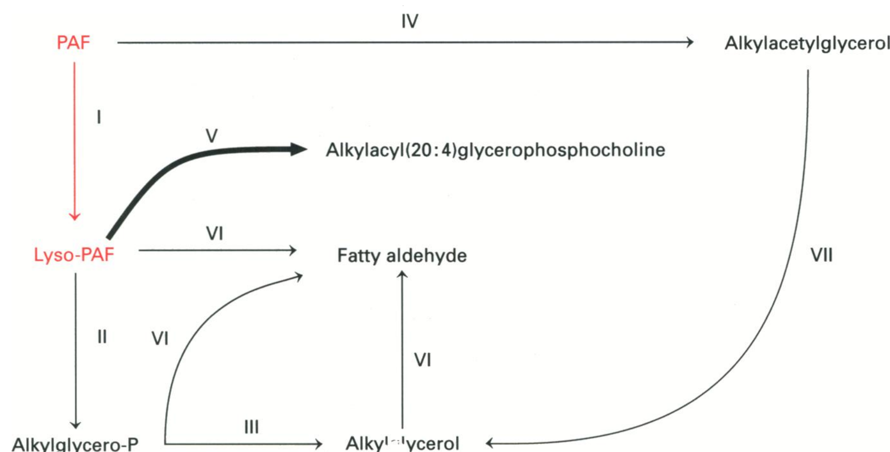



**Figure 2 Catabolic reactions involving PAF and its metabolites**

The Roman numerals designate the following enzymes: I, PAF acetylhydrolase; II, lysophospholipase D; III, alkylglycero-P phosphohydrolase; IV, phospholipase C; V, CoA-independent transacylase (reacylation of lyso-PAF); VI, *O*-alkyl cleavage enzyme (mono-oxygenase); and VII, alkylacetylglycerol acetylhydrolase. The primary reaction responsible for the inactivation of PAF is shown in red; the major mechanism for the removal of lyso-PAF is the reacylation reaction designated by the bold arrow.

lacking about the quantitative contribution of lysophospholipases I and II in the catabolism of PAF and its related analogues.

## CONCLUDING REMARKS

The metabolic pathways illustrated in Figures 1 and 2 depict the overall integrated sequence of reactions known to be involved in the biosynthesis and catabolism of PAF and related lipids; these charts are intended to show how the various enzymes described in this review article relate to each other. Except for PAF acetylhydrolase, none of the other enzymes directly related to PAF metabolism have been purified to homogeneity. This is undoubtedly due to difficulties in solubilizing these enzyme activities from the membrane environment where they reside in order to proceed with the currently available conventional chromatographic approaches for enzyme purification. However, in spite of such problems, alternative molecular biology techniques for obtaining the purified forms of these membrane-associated enzymes of PAF metabolism via cloning techniques appear promising. Only after this difficult task of enzyme purification is accomplished will our understanding of the precise molecular enzymic mechanisms and regulatory controls be forthcoming. Such information will not only provide a more complete biochemical understanding of the mode of action of PAF but also could lead to novel new approaches of pharmacological intervention that target specific biosynthetic or catabolic enzymes involved in PAF-linked pathological processes.

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## REFERENCES

1 Sturk, A., ten Cate, J. W., Hosford, D., Mencia-Huerta, J. M. and Braquet, P. (1989) Adv. Lipid Res. **23**, 219–276
2 Snyder, F. (1990) Am. J. Physiol. (Cell Physiol.) **259**, C697–C708
3 Prescott, S. M., Zimmerman, G. A. and McIntyre, T. M. (1990) J. Biol. Chem. **265**, 17381–17384
4 Venable, M. E., Zimmerman, G. A., McIntyre, T. M. and Prescott, S. M. (1993) J. Lipid Res. **34**, 691–702
5 Chao, W. and Olson, M. S. (1993) Biochem. J. **292**, 617–629
6 Snyder, F. (1995) Biochim. Biophys. Acta, in the press
7 MacDonald, J. I. S. and Sprecher, H. (1991) Biochim. Biophys. Acta **1084**, 105–121
8 Snyder, F., Lee, T.-c. and Blank, M. L. (1992) Prog. Lipid Res. **31**, 65–86
9 Uemura, Y., Lee, T.-c. and Snyder, F. (1991) J. Biol. Chem. **266**, 8268–8272
10 Nieto, M. L., Venable, M. E., Bauldry, S. A., Greene, D. G., Kennedy, M., Bass, D. A. and Wykle, R. L. (1991) J. Biol. Chem. **266**, 18699–18706
11 Lee, T.-c., Uemura, Y. and Snyder, F. (1992) J. Biol. Chem. **267**, 19992–20001
12 Ou, M.-c., Malone, B., Lee, T.-c. and Snyder, F. (1993) FASEB J. **7**, A1265
13 Ou, M.-c., Lee, T.-c. and Snyder, F. (1994) FASEB J. **8**, A1402
14 Stafforini, D. M., Prescott, S. M. and McIntyre, T. M. (1987) J. Biol. Chem. **262**, 4223–4230
15 Stafforini, D. M., Rollins, E. N., Prescott, S. M. and McIntyre, T. M. (1993) J. Biol. Chem. **268**, 3857–3865
16 Hattori, M., Arai, H. and Inoue, K. (1993) J. Biol. Chem. **268**, 18748–18753
17 Snyder, F., Lee, T.-c. and Wykle, R. L. (1985) in The Enzymes of Biological Membranes (Martonosi, A. N., ed.), vol. 2, pp. 1–58, Plenum, New York
18 Snyder, F., Malone, B. and Wykle, R. L. (1969) Biochem. Biophys. Res. Commun. **34**, 40–47
19 Wykle, R. L. and Snyder, F. (1969) Biochem. Biophys. Res. Commun. **37**, 658–662
20 Snyder, F., Blank, M. L., Malone, B. and Wykle, R. L. (1970) J. Biol. Chem. **245**, 1800–1805
21 Snyder, F., Malone, B. and Blank, M. L. (1970) J. Biol. Chem. **245**, 1790–1799
22 Snyder, F., Rainey, W. T., Jr., Blank, M. L. and Christie, W. H. (1970) J. Biol. Chem. **245**, 5853–5856
23 Hajra, A. K. (1969) Biochem. Biophys. Res. Commun. **37**, 486–492
24 Hajra, A. K. (1970) Biochem. Biophys. Res. Commun. **39**, 1037–1044
25 Wykle, R. L., Piantadosi, C. and Snyder, F. (1972) J. Biol. Chem. **247**, 2944–2948
26 Brown, A. J. and Snyder, F. (1992) Methods Enzymol. **209**, 377–384
27 Horie, S., Das, A. K. and Hajra, A. K. (1992) Methods Enzymol. **209**, 385–390
28 Snyder, F., Clark, M. and Piantadosi, C. (1973) Biochem. Biophys. Res. Commun. **53**, 350–356
29 Hajra, A. K., Jones, C. L. and Davis, P. A. (1978) Adv. Exp. Med. Biol. **101**, 369–378
30 Bell, O. E., Jr., Blank, M. L. and Snyder, F. (1971) Biochim. Biophys. Acta **231**, 579–583
31 Ellingboe, J. and Karnovsky, M. L. (1967) J. Biol. Chem. **242**, 5693–5699
32 Friedberg, S. J. and Greene, R. C. (1967) J. Biol. Chem. **242**, 5709–5714
33 Wood, R., Walton, M., Healy, K. and Cumming, R. B. (1970) J. Biol. Chem. **245**, 4276–4285
34 Wykle, R. L. and Snyder, F. (1970) J. Biol. Chem. **245**, 3047–3058
35 Stoffel, W. and LeKim, D. (1971) Hoppe-Seyler's Z. Physiol. Chem. **352**, 501–511
36 Friedberg, S. J., Heifetz, A. and Green, R. C. (1971) J. Biol. Chem. **246**, 5822–5827
37 Friedberg, S. J., Heifetz, A. and Green, R. C. (1972) Biochemistry **11**, 297–301
38 Friedberg, S. J. and Heifetz, A. (1973) Biochemistry **12**, 1100–1106
39 Brown, A. J. and Snyder, F. (1983) J. Biol. Chem. **258**, 4184–4189
40 Friedberg, S. J. and Alkek, R. D. (1977) Biochemistry **16**, 5291–5294
41 Davis, P. A. and Hajra, A. K. (1979) J. Biol. Chem. **254**, 4760–4763
42 Brown, A. J. and Snyder, F. (1982) J. Biol. Chem. **257**, 8835–8839
43 Brown, A. J., Glish, G. L., McBay, E. H. and Snyder, F. (1985) Biochemistry **24**, 8012–8016
44 Friedberg, S. J., Weintraub, S. T., Singer, M. R. and Greene, R. C. (1983) J. Biol. Chem. 258, 136–142
45 Jones, C. L. and Hajra, A. K. (1977) Biochem. Biophys. Res. Commun. **76**, 1183–1143
46 Jones, C. L. and Hajra, A. K. (1980) J. Biol. Chem. **255**, 8289–8295
47 Hajra, A. K., Burke, C. L. and Jones, C. L. (1979) J. Biol. Chem. **254**, 10896–10900
48 Hajra, A. K. and Bishop, J. E. (1982) Ann. N. Y. Acad. Sci. **386**, 170–182
49 Bishop, J. E., Salem, M. and Hajra, A. K. (1982) Ann. N. Y. Acad. Sci. **386**, 411–413
50 Schrakamp, G., Roosenboom, C. F. P., Schutgens, R. B. H., Warders, R. J. A., Heymans, H. S. A., Tager, J. M. and van den Bosch, H. (1985) J. Lipid Res. **26**, 867–873
51 Datta, N. S., Wilson, G. N. and Hajra, A. K. (1984) N. Engl. J. Med. **311**, 1080–1083
52 Davis, P. A. and Hajra, A. K. (1977) Biochem. Biophys. Res. Commun. **74**, 100–105
53 Friedberg, S. J., Gomillion, D. M. and Stotter, P. L. (1980) J. Biol. Chem. **255**, 1074–1079
54 Brown, A. J. and Snyder, F. (1980) Fed. Proc. **39**, 1993
55 Davis, P. A. and Hajra, A. K. (1981) Arch. Biochem. Biophys. **211**, 20–29
56 Zomer, A. W. M., de Weerd, W. F. C., Langeveld, J. and van den Bosch, H. (1993) Biochim. Biophys. Acta **1170**, 189–196
57 Hixson S. and Wolfenden, R. (1981) Biochem. Biophys. Res. Commun. **101**, 1064–1070
58 Hajra, A. K. (1968) J. Biol. Chem. **243**, 3458–3465
59 Hajra, A. K. and Agranoff, B. W. (1968) J. Biol. Chem. **243**, 1617–1622
60 Hajra, A. K., Seguin, E. B. and Agranoff, B. W. (1968) J. Biol. Chem. **243**, 1609–1616.
61 Webber, K. O. and Hajra, A. K. (1993) Arch. Biochem. Biophys. **300**, 88–97
62 Hajra, A. K. (1983) in Ether Lipids. Biochemical and Biomedical Aspects (Mangold, H. K. and Paltauf, F., eds.), pp. 85–106, Academic Press, New York
63 LaBelle, E. F. Jr. and Hajra, A. K. (1972) J. Biol. Chem. **247**, 5825–5834
64 Chae, K., Piantadosi, C. and Snyder, F. (1973) J. Biol. Chem. **248**, 6718–6723
65 LaBelle, E. F. Jr. and Hajra, A. K. (1974) J. Biol. Chem. **249**, 6936–6944
66 Chae, K., Piantadosi, C. and Snyder, F. (1973) Biochem. Biophys. Res. Commun. **51**, 119–124
67 Rock, C. O. and Snyder, F. (1974) J. Biol. Chem. **249**, 5382–5387
68 Snyder, F., Blank, M. L. and Malone, B. (1970) J. Biol. Chem. **245**, 4016–4018
69 Lee, T.-c., Blank, M. L., Fitzgerald, V. and Snyder, F. (1982) Biochim. Biophys. Acta **713**, 479–483
70 Radominska-Pyrek, A., Strosznajder, J., Dabrowiecki, Z., Goracci, G., Chojnacki, T. and Horrocks, L. A. (1977) J. Lipid Res. **18**, 53–58
71 Blank, M. L., Wykle, R. L., Alper, S. and Snyder, F. (1974) Biochim. Biophys. Acta **348**, 397–403
72 Kawasaki, T. and Snyder, F. (1988) J. Biol. Chem. **263**, 2593–2596
73 Horrocks, L. A. (1972) in Ether Lipids Chemistry and Biology (Snyder, F., ed.), pp. 177–272, Academic Press, New York
74 Wykle, R. L., Blank, M. L., Malone, B. and Snyder, F. (1972) J. Biol. Chem. **247**, 5442–5447
75 Paltauf, F. and Holasek, A. (1973) J. Biol. Chem. **248**, 1609–1615

76 Blank, M. L., Wykle, R. L. and Snyder, F. (1972) Biochem. Biophys. Res. Commun. **47**, 1203–1208
77 Wykle, R. L. and Schremmer-Lockmiller, J. M. (1975) Biochim. Biophys. Acta **380**, 291–298
78 Paltauf, F., Prough, R. A., Masters, B. S. S. and Johnston, J. M. (1974) J. Biol. Chem. **249**, 2661–2662
79 Lee, T.-c., Wykle, R. L., Blank, M. L. and Snyder, F. (1973) Biochem. Biophys. Res. Commun. **55**, 574–579
80 Holloway, P. W. and Katz, J. T. (1972) Biochemistry **11**, 3689–3695
81 Oshino, N. and Omura, T. (1973) Arch. Biochem. Biophys. **157**, 399–404
82 Blank, M. L., Fitzgerald, V., Lee, T.-c. and Snyder, F. (1993) Biochim. Biophys. Acta **1166**, 309–312
83 Strum, J. C. and Daniel, L. W. (1993) J. Biol. Chem. **268**, 25500–25508
84 Ford, D. A. and Gross, R. W. (1994) Biochemistry **33**, 1216–1222
85 Alonso, F., Henson, P. M. and Leslie, C. C. (1986) Biochim. Biophys. Acta **878**, 273–280
86 Angle, M. J., Paltauf, F. and Johnston, J. M. (1988) Biochim. Biophys. Acta **962**, 234–240
87 Channon, J. Y. and Leslie, C. C. (1990) J. Biol. Chem. **265**, 5409–5413
88 Clark, J. D., Milona, N. and Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7708–7712
89 de Carvalho, M. S., McCormack, F. X. and Leslie, C. C. (1993) Arch. Biochem. Biophys. **306**, 534–540
90 Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13060
91 Diez, E. and Mong, S. (1990) J. Biol. Chem. **265**, 14654–14661
92 Gronich, J. H., Bonventre, J. V. and Nemenoff, R. A. (1990) Biochem. J. **271**, 37–43
93 Hazen, S. L., Stuppy, R. J. and Gross, R. W. (1990) J. Biol. Chem. **265**, 10622–10630
94 Hazen, S. L., Ford, D. A. and Gross, R. W. (1991) J. Biol. Chem. **266**, 5629–5633
95 Hazen, S. L., Hall, C. R., Ford, D. A. and Gross, R. W. (1993) J. Clin. Invest. **91**, 2513–2522
96 Kim, D. K., Kudo, I. and Inoue, K. (1991) Biochim. Biophys. Acta **1083**, 80–88
97 Kramer, R. M., Roberts, E. F., Manetta, J. and Putnam, J. E. (1991) J. Biol. Chem. **266**, 5268–5272
98 Leslie, C. C. (1991) J. Biol. Chem. **266**, 11366–11371
99 Leslie, C. C., Voelker, D. R., Channon, J. Y., Wall, M. M. and Zelarney, P. T. (1988) Biochim. Biophys. Acta **963**, 476–492
100 Wijkander, J. and Sundler, R. (1991) Eur. J. Biochem. **202**, 873–880
101 Wolf, R. A. and Gross, R. W. (1985) J. Biol. Chem. **260**, 7295–7303
102 Wong, B., Tang, W. and Ziboh, V. A. (1992) FEBS Lett. **305**, 213–216
103 Shikano, M., Masuzawa, Y., Yazawa, K., Takayama, K., Kudo, I. and Inoue, K. (1994) Biochim. Biophys. Acta **1212**, 211–216
104 Diez, E., Chilton, F. H., Stroup, G., Mayer, R. J., Winkler, J. D. and Fonteh, A. N. (1994) Biochem. J. **301**, 721–726
105 Blank, M. L., Smith, Z. L., Fitzgerald, V. and Snyder, F. (1995) Biochim. Biophys. Acta, in the press
106 Sugiura, T., Fukuda, T., Masuzawa, Y. and Waku, K. (1990) Biochim. Biophys. Acta. **1047**, 223–232
107 Venable, M. E., Nieto, M. L., Schmitt, J. D. and Wykle, R. L. (1991) J. Biol. Chem. **266**, 18691–18698
108 Venable, M. E., Olson, S. C., Nieto, M. L. and Wykle, R. L. (1993) J. Biol. Chem. **268**, 7965–7975
109 Winkler, J. D., Sung, C.-M., Hubbard, W. C. and Chilton, F. H. (1992) Biochem. Pharmacol. **44**, 2055–2066
110 Colard, O., Bidault, J., Berton, M. and Ninio E. (1993) Eur. J. Biochem. **216**, 835–840
111 Suga, K., Kawasaki, T., Blank, M. L. and Snyder, F. (1990) J. Biol. Chem. **265**, 12363–12371
112 Ramesha, C. S. and Pickett, W. C. (1986) J. Biol. Chem. **261**, 7592–7595
113 Tessner, T. G., Greene, D. G. and Wykle, R. L. (1990) J. Biol. Chem. **265**, 21032–21038
114 Winkler, J. D., Sung, C.-M., Bennett, C. F. and Chilton, F. H. (1991) Biochim. Biophys. Acta **1081**, 339–346.
115 Winkler, J. D., McCarte-Roshak, A., Huang, L., Sung, C.-M., Bolognese, B. and Marshall, L. A. (1994) J. Lipid Med. Cell Signalling 10, 315–330
116 Lee, T.-c., Vallari, D. S. and Snyder, F. (1992) Methods Enzymol. **209**, 396–401
117 Wykle, R. L., Malone, B. and Snyder, F. (1980) J. Biol. Chem. **255**, 10256–10260
118 Lee, T.-c. and Snyder, F. (1985) in Phospholipids and Cellular Regulation (Kuo, J. F., ed.), vol. 1, pp. 1–39, CRC Press, Boca Raton
119 Ribbes, G., Ninio, E., Fontan, P., Record, M., Chap, H., Benveniste, J. and Douste-Blazy, L. (1985) FEBS Lett. **191**, 195–199
120 Mollinedo, F., Gomez-Cambronero, J., Cano, K. and Sanchez-Crespo, M. (1988) Biochem. Biophys. Res. Commun. **154**, 1232–1239
121 Record, M. and Snyder, F. (1990) J. Lip. Med. 2, 1–8.
122 Ribbes, G., Gelas, P., Lumb, R., Record, M., Terce, F. and Chap, H. (1991) J. Lipid Med. **4**, 251–264
123 Record, M., Ribbes, G., Terce, F. and Chap, H. (1989) J. Cell. Biochem. **40**, 353–359
124 Lee, T.-c. (1985) J. Biol. Chem. **260**, 10952–10955
125 Ninio, E., Mencia-Huerta, M., Heymans, F. and Benveniste, J. (1982) Biochim. Biophys. Acta **710**, 23–31
126 Tessner, T. G. and Wykle, R. L. (1987) J. Biol. Chem. **262**, 12660–12664
127 Holland, M. R., Venable, M. E., Wahtley, R. E., Zimmerman, G. A., McIntyre, T. M. and Prescott, S. M. (1992) J. Biol. Chem. **267**, 22883–22890
128 Remy, E., Lenoir, G., Houben, A., Vandesteene, C. and Remacle, J. (1989) Biochim. Biophys. Acta **1005**, 87–92
129 Garcia, M. C., Fernandez-Gallardo, S., Gijon, M. A., Garcia, C., Nieto, M. L. and Sanchez-Crespo, M. (1990) Biochem. J. **268**, 91–98
130 Ninio, E., Joly, F. and Bessou, G. (1988) Biochim. Biophys. Acta **963**, 288–294
131 Seyama, K. and Ishibashi, T. (1987) Lipids **22**, 185–189
132 Gomez-Cambronero, J., Velasco, S., Sánchez–Crespo, M., Vivanco, F. and Mato, J. M. (1986) Biochem. J. **237**, 439–445
133 Lenihan, D. J. and Lee, T.-c. (1984) Biochem. Biophys. Res. Commun. **120**, 834–839
134 Gomez-Cambronero, J., Velasco, S., Mato, J. M. and Sanchez-Crespo, M. (1985) Biochim. Biophys. Acta **845**, 516–519
135 Gomez-Cambronero, J., Mato, J. M., Vivanco, F. and Sanchez-Crespo, M. (1987) Biochem. J. **245**, 893–898
136 Nieto, M. L., Velasco, S. and Sanchez-Crespo, M. (1988) J. Biol. Chem. **263**, 4607–4611
137 Domenech, C., Machado-De Domenech, E. and Soling, H.-D. (1987) J. Biol. Chem. **262**, 5671–5676
138 Ninio, E., Joly, F., Hieblot, C., Bessou, G., Mencia-Huerta, J. M. and Benveniste, J. (1987) J. Immunol. **139**, 154–160
139 Leyravaud, S., Bossant, M.-J., Joly, F., Bessou, G., Benveniste, J. and Ninio, E. (1989) J. Immunol. **143**, 245–249
140 Liu, M. and Subbaiah, P. V. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 6035–6039
141 Lee, T.-c., Malone, B. and Snyder, F. (1986) J. Biol. Chem. **261**, 5373–5377
142 Lee, T.-c., Malone, B. and Snyder, F. (1988) J. Biol. Chem. **263**, 1755–1760
143 Renooij, W. and Snyder, F. (1981) Biochim. Biophys. Acta **663**, 545–556
144 Woodard, D. S., Lee, T.-c. and Snyder, F. (1987) J. Biol. Chem. **262**, 2520–2527
145 Lee, T.-c., Malone, B. and Snyder, F. (1990) in Proceedings of the Taipei Satellite Symposium of Platelet Activating Factor (Hicks, N., ed.), pp. 1–4, Excerpta Medica, Hong Kong
146 Heller, R., Bussolino, F., Ghigo, D., Garbarino, G., Pescarmona, G., Till, U. and Bosia, A. (1991) J. Biol. Chem. **266**, 21358–21361
147 Baker, R. R. and Chang, H.-y. (1994) Biochim. Biophys. Acta **1213**, 27–33
148 Snyder, F. and Lee, T.-c. (1992) Methods Enzymol. **209**, 230–234
149 McNamara, M. J. C., Schmitt, J. D., Wykle, R. L. and Daniel, L. W. (1984) Biochem. Biophys. Res. Commun. **122**, 824–830
150 Bass, D. A., McPhail, L. C., Schmitt, J. D., Morris-Natschceke, S., McCall, C. E. and Wykle, R. L. (1989) J. Biol. Chem. **264**, 19610–19617
151 Lee, T.-c. and Snyder, F. (1992) Methods Enzymol. **209**, 279–283
152 Blank, M. L., Lee, Y. J., Cress, E. A. and Snyder, F. (1988) J. Biol. Chem. **263**, 5656–5661
153 Vallari, D. S., Record, M. and Snyder, F. (1990) Arch. Biochem. Biophys. **276**, 538–545
154 Lee, T.-c., Malone, B., Blank, M. L., Fitzgerald, V. and Snyder F. (1990) J. Biol. Chem. **265**, 9181–9187
155 Francescangeli, E. and Goracci G. (1989) Biochem. Biophys. Res. Commun. **161**, 107–112
156 Bussolino, F., Gremo, F., Tetta, C., Pescarmona, G. P. and Camussi, G. (1986) J. Biol. Chem. **261**, 16502–16508
157 Bussolino, F., Pescarmona, G., Camussi, G. and Gremo, F. (1988) J. Neurochem. **51**, 1755–1759
158 Nieto, M. L., Velasco, S. and Sanchez-Crespo, M. (1988) J. Biol. Chem. **263**, 2217–2222
159 Camussi, G., Tetta, C., Bussolino, F. and Baglioni, C. (1989) Eur. J. Biochem. **182**, 661–666
160 Lee, T.-c., Malone, B., Woodard, D. and Snyder, F. (1989) Biochem. Biophys. Res. Commun. **163**, 1002–1005
161 Kent, C. (1990) Prog. Lipid Res. **29**, 87–105
162 Vance, D. (1989) in Phosphatidylcnoline Metabolism (Vance, D., ed.), pp. 33–45, CRC Press, Boca Raton
163 Tijburg, L. B. M., Geelen, M. J. H. and van Golde, L. M. G. (1989) Biochim. Biophys. Acta **1004**, 1–19

164 Lee, T.-c., Blank, M. L., Fitzgerald, V., Malone, B. and Snyder, F. (1987) in New Horizons in Platelet Activating Factor Research (Winslow, C. M. and Lee, M. L., eds.), pp. 37–44, John Wiley and Sons, Chichester
165 Blank, M. L., Lee, T.-c., Fitzgerald, V. and Snyder, F. (1981) J. Biol. Chem. **256**, 175–178
166 Stafforini, D. M., Prescott, S. M., Zimmerman, G. A. and McIntyre, T. M. (1991) Lipids **26**, 979–985
167 Cabot, M. C., Faulkner, L. A., Lackey, R. J. and Snyder, F. (1984) Comp. Biochem. Physiol. **78B**, 37–40
168 Lenihan, D. J., Greenberg, N. and Lee, T.-c. (1985) Comp. Biochem. Physiol. **81C**, 81–86
169 Lambremont, E. N., Malone, B. and Snyder, F. (1988) Arch. Insect Biochem. Physiol. **7**, 37–45
170 Tselepis, A. D., Lekka, M. E. and Tsoukatos, D. (1991) FEBS Lett. **288**, 147–150
171 Farr, R. S., Cox, C. P., Wardlow, M. L. and Jorgensen, R. (1980) Clin. Immunol. Immunopathol. **5**, 318–330
172 Farr, R. S., Wardlow, M. L., Cox, C. P., Meng, K. E. and Greene, D. E. (1983) Federation Proc. **42**, 3120–3122
173 Wardlow, M. L., Cox, C. P., Meng, K. E., Greene, D. E. and Farr, R. S. (1986) J. Immunol. **136**, 3441–3446
174 Blank, M. L., Hall, M. N., Cress, E. A. and Snyder, F. (1983) Biochem. Biophys. Res. Commun. **113**, 666–671
175 Stafforini, D. M., McIntyre, T. M., Carter, M. E. and Prescott, S. M. (1987) J. Biol. Chem. **262**, 4215–4222
176 Stafforini, D. M., Carter, M. E., Zimmerman, G. A., McIntyre, T. M. and Prescott, S. M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 2393–2397
177 Lee, T.-c., Fitzgerald, V., Chatterjee, R., Malone, B. and Snyder, F. (1994) J. Lipid Med. **9**, 267–283
178 Yanoshita, R., Kudo, I., Ikizawa, K., Chang, H. W., Kobayashi, S., Ohno, M., Nojima, S. and Inoue, K. (1988) J. Biochem. **103**, 815–819
179 Stafforini, D. M., McIntyre, T. M. and Prescott, S. M. (1990) Methods Enzymol. **187**, 344–357
180 Snyder, F., Blank, M. L., Lee, T.-c., Robinson, M. and Woodard, D. (1987) Methods Enzymol. **141**, 379–396
181 Steinbrecher, U. P. and Pritchard, P. H. (1989) J. Lipid Res. **30**, 305–315
182 Stremler, K. E., Stafforini, D. M., Prescott, S. M., Zimmerman, G. A.. and McIntyre, T. M. (1989) J. Biol. Chem. **264**, 5331–5334
183 Stremler, K. E., Stafforini, D. M., Prescott, S. M. and McIntyre, T. M. (1991) J. Biol. Chem. **266**, 11095–11103
184 Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. and Steinberg, D. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 3883–3887
185 Parthasarathy, S., Steinbrecher, U. P., Barnett, J., Witztum, J. L. and Steinberg, D. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 3000–3004
186 Suzuki, Y., Miwa, M., Harada, M. and Matsumoto, M. (1988) Eur. J. Biochem. **172**, 117–120
187 Korth, R., Bidault, J., Palmantier, R., Benveniste, J. and Ninio, E. (1993) Lipids **28**, 193–199
188 Tarbet, E. B., Stafforini, D. M., Elstad, M. R., Zimmerman, G. A., McIntyre, T. M. and Prescott, S. M. (1991) J. Biol. Chem. **286**, 16667–16673
189 Satoh, K., Imaizumi, T. A., Kawamura, Y., Yoshida, H., Hiramoto, M., Takamatsu, S. and Takamatsu, M. (1991) J. Clin. Invest. **87**, 476–481
190 Palmantier, R., Dulioust, A., Maiza, H., Benveniste, J. and Ninio, E. (1989) Biochem. Biophys. Res. Commun. **162**, 475–482
191 Elstad, M. R., Stafforini, D. M., McIntyre, T. M., Prescott, S. M. and Zimmerman, G. A. (1989) J. Biol. Chem. **264**, 8467–8470
192 Narahara, H., Frenkel, R. A. and Johnston, J. M. (1993) Arch. Biochem. Biophys. **301**, 275–281
193 Satoh, K., Imaizumi, T. A., Yoshida, H. and Takamatsu, S. (1993) Metabolism **42**, 672–677
194 Satoh, K., Imaizumi, T. A., Yoshida, H. and Takamatsu, S. (1994) J. Lab. Clin. Med. **123**, 225–231
195 Narahara, H. and Johnston, J. M. (1993) Am. J. Obstet. Gynecol. **169**, 1321–1326
196 Narahara, H. and Johnston, J. M. (1993) Am. J. Obstet. Gynecol. **169**, 531–537
197 Blank, M. L., Smith, Z. L., Cress, E. A. and Snyder, F. (1990) Biochim. Biophys. Acta **1042**, 153–158
198 Tietz, A., Lindberg, M. and Kennedy, E. P. (1964) J. Biol. Chem. **239**, 4081–4090
199 Kaufman, S. (1959) J. Biol. Chem. **234**, 2677–2682
200 Soodsma, J. F., Piantadosi, C. and Snyder, F. (1972) J. Biol. Chem. **247**, 3923–3929
201 Lee, T.-c., Blank, M. L., Fitzgerald, V. and Snyder, F. (1981) Arch. Biochem. Biophys. **208**, 353–357
202 Snyder, F., Malone, B. and Piantadosi, C. (1973) Biochem. Biophys. Acta **316**, 259–265
203 Hoffman, D. R., Hoffman, L. H. and Snyder, F. (1986) Cancer Res. **46**, 5803–5809
204 Pfleger, R. C., Piantadosi, C. and Snyder, F. (1967) Biochim. Biophys. Acta **144**, 633–648
205 Snyder, F., Hibbs, M. and Malone, B. (1971) Biochim. Biophys. Acta **231**, 409–411
206 Wykle, R. L. and Schremmer, J. M. (1974) J. Biol. Chem. **259**, 1742–1746
207 Wykle, R. L., Kraemer, W. F. and Schremmer, J. M. (1977) Arch. Biochem. Biophys. **184**, 149–155
208 Wykle, R. L., Kraemer, W. F. and Schremmer, J. M. (1980) Biochim. Biophys. Acta **619**, 58–67
209 Kawasaki, T. and Snyder, F. (1987) Biochim. Biophys. Acta **920**, 85–93
210 Nishihira, J. and Ishibashi, T. (1986) Lipids **21**, 780–785
211 Okayasu, T., Hoshii, K., Seyama, K., Ishibashi, T. and Iami, Y. (1986) Biochim. Biophys. Acta **876**, 58–64
212 Sturk, A., Schaap, M. C. L., Prins, A., ten Cate, J. W. and van den Bosch, H. (1989) Biochim. Biophys. Acta **993**, 148–156
213 Aarsman, A. J., Neys, F. W. and van den Bosch, H. (1991) J. Biochem. **200**, 187–193
214 Triggiani, M., D'Souza, D. M. and Chilton, F. H. (1991) J. Biol. Chem. **266**, 6928–6935
215 Triggiani, M., Fonteh, A. N. and Chilton, F. H. (1992) Biochem. J. **286**, 497–503
216 van den Bosch, H., Sturk, A., ten Cate, J. W. and Aarsman, A. J. (1991) Lipids **26**, 967–973