Ether-linked lipids and their bioactive species

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1. Introduction

Naturally occurring ether lipids contain either O-alkyl or O-alk-1-enyl groupings. Those possessing the O-alk-1-envl moiety with a cis double bond adjacent to the ether bridge are referred to as plasmalogens, as well as vinyl ethers. Both the O-alkyl and O-alk-1-envl substituents are generally located at the sn-1 position of the glycerol moiety although di- and tetra-O-alkylglycerolipids have been described in some cells. Unlike the diverse types of acyl moieties present in glycerolipids, the predominant O-alkyl and O-alk-1-enyl ether-linked chains generally consist mainly of 16:0, 18:0, and 18:1 aliphatic groupings, but other types of chain lengths, degrees of unsaturation, and occasional branched-chains do exist as minor components. Except for intermediary metabolites and certain bioactive lipids, ether linkages in phospholipids of mammalian cells exist almost exclusively in the choline and ethanolamine glycerolipid classes. The majority of the O-alkyl moieties normally occur as plasmanylcholines¹, whereas the O-alk-1-enyl grouping is mainly associated with the plasmenylethanolamines with the exception of heart where plasmenylcholines predominate. Some neutral lipids such as alkyldiacylglycerols (glyceryl ether diesters) and alkylacylglycerols, analogs of triacylglycerols and diacylglycerols, respectively, are also found in cells. Fig. 1 illustrates the chemical structures of the most common ether lipids found in mammals.

A number of books [1-6] and review articles [7-14] on ether lipids, some specifically emphasizing platelet-activating factor (PAF), are recommended as reading material. These sources provide a comprehensive listing of published papers.

2. Historical highlights

The early literature concerning ether-linked lipids has also been covered in detail [1,2,9,15]. Perhaps the first evidence, albeit circumstantial, to suggest the existence of O-alkyl lipids in nature was the isolation of an unsaponifiable fraction of lipids from starfish that was referred to as 'astrol', which was subsequently shown to have similar

¹ Plasmanyl designates the radical '1-alkyl-2-acyl-*sn*-glycero-3-phospho-', whereas plasmenyl represents the radical '1-alk-1-enyl-2-acyl-*sn*-glycero-3-phospho-'; the prefix phosphatidyl is used only to denote the radical '1,2-diacyl-*sn*-glycero-3-phospho-'. 'Radyl' is used as a prefix in glycerolipid nomenclature when the aliphatic substituents are unknown at the *sn*-positions of the glycerol moiety or when either acyl, alkyl, or alk-1-enyl moieties would be of equal importance.



Fig. 1. Chemical structures of biologically significant types of ether-linked lipids found in mammalian cells.

properties to batyl alcohol, an alkylglycerol possessing an 18-carbon aliphatic chain at the *sn*-1 position of the glycerol moiety. During the same period the presence of alkyl ether lipids in liver oils of various saltwater fish was described by the Japanese scientists M. Tsujimoto and Y. Toyama (1922). The common names of the alkylglycerols, *chimyl* [16:0 alkyl], *batyl* [18:0 alkyl], and *selachyl* [18:1 alk-9-enyl] alcohols, are based on the fish species from which they were originally isolated. Complete proof of the precise chemical nature of the alkyl linkage at the *sn*-1 position in these glycerolipids was provided by W.H. Davies, I.M. Heilbron, and W.E. Jones (1933) from England.

The German scientists, R. Feulgen and K. Voit (1924) originally described plasmalogens in a variety of fresh tissue slices preserved in a HgCl₂ solution after being erroneously treated with a fuchsin–sulfurous acid reagent without the normal fixation and related histological processing with organic solvents. Only the cytoplasm of cells, but not the nuclei, was stained a red–violet color, which led to the conclusion that an aldehyde was present in the cell plasma. This substance was called 'plasmal'. If the histological preparations were treated with a lipid-extracting solvent before exposure to the dye, no colored stain appeared in the cytoplasm. This unknown precursor of the cytosolic aldehyde that reacted with the dye was called plasmalogen, a name still retained as the generic term for all alk-1-enyl-containing glycerolipid classes.

It was not until the 1950s that the precise chemical structure of the alk-1-enyl linkage in ethanolamine plasmalogens was proven, primarily through the combined efforts of M.M. Rapport and G.V. Marinetti in the United States, G.M. Gray in England, E. Klenk and H. Debuch in Germany, and their various co-workers. The first cell-free systems to synthesize the alkyl ether bond were described independently in 1969 by F. Snyder, R.L. Wykle, and B. Malone and by A. Hajra. Shortly thereafter, studies by R.L. Wykle, M.L. Blank, B. Malone, and F. Snyder and by F. Paltauf and A. Holasek demonstrated that the *O*-alkyl moiety of an intact phospholipid could be enzymatically desaturated to the alk-1-enyl grouping (see Section 6.2.5). One of the most significant developments in the ether-lipid field occurred in 1979 when one of the most potent bioactive molecules known, an acetylated form of a choline-containing alkylglycerolipid called platelet-activating factor or PAF, was identified independently by three separate groups.

3. Natural occurrence

Chemical, chromatographic, and mass spectral methods for analyzing ether-linked glycerolipids have been reviewed [16,17]. Ether-linked phospholipids generally are isolated as a mixture with their ester-linked counterparts.

Ether-linked lipids occur throughout the animal kingdom and are even found as minor components in several higher plants. Some mammalian tissues, and avian, marine, molluscan, protozoan, and bacterial lipid extracts contain significant proportions of ether-linked lipids. Highest levels of ether lipids in mammals occur in nervous tissue, heart muscle, testes, kidney, preputial glands, tumor cells, erythrocytes, bone marrow, spleen, skeletal tissue, neutrophils, eosinophils, macrophages, platelets, and lipoproteins. The large quantities of ethanolamine plasmalogens associated with various lipoproteins from rat serum and human plasma (36% and 50%, respectively, of the total ethanolamine phosphatides) is of particular interest since the liver contains relatively low amounts of ether lipids and the plasmalogens are not acquired in the lipoproteins after their secretion. Although the dietary consumption of ether lipids by humans has largely been ignored by nutritionists, it is clear that certain meats and seafoods can contain relatively high amounts of these lipids.

Analogs of triacylglycerols have also been described. 1-Alkyl-2,3-diacyl-*sn*-glycerols are characteristically elevated in tumor lipids and 1-alk-1-enyl-2,3-diacyl-*sn*-glycerols (neutral plasmalogens) have also been detected in tumors and adipose tissue of mammals and in fish liver oil. In fact, even alkylacetylacylglycerols have been shown to be formed by human leukemic cells.

1-Alkyl-2-acyl-*sn*-glycero-3-phosphocholine (Fig. 1), a significant component of platelets, neutrophils, macrophages, eosinophils, basophils, monocytes, and endothelial, mast, and HL-60 cells (a human promyelocytic leukemic cell line), is a precursor of platelet-activating factor (PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; see Fig. 2). Thus, this precursor appears to be a constituent of all cells known to produce



Fig. 2. Chemical structures of PAF and structurally related ether-linked glycerolipids possessing biological activities.

PAF by the remodeling pathway; in human neutrophils and eosinophils the alkyl subclass comprises 45 and 70 mol% of the choline-linked phosphoglycerides, respectively, while the ethanolamine-linked class contains 60–65 mol% plasmalogen. PAF is also found in saliva, urine, and amniotic fluid, which indicates that other cell types could be the source of PAF in these fluids.

Dialkylglycerophosphocholines have been reported as minor constituents of bovine heart and spermatozoa. Moreover, heart tissue is unique with respect to its plasmalogen content, since in some animal species, this is the only mammalian tissue known to contain significant amounts of choline plasmalogens instead of the usually encountered ethanolamine plasmalogens.

Halophilic bacteria contain an unusual dialkyl type of glycerolipid (a diphytanyl ether analog of phosphatidylglycerophosphate) that has an opposite stereochemical configuration from all other known ether-linked lipids, i.e., the ether linkages are located at the sn-2 and sn-3 positions. The biosynthetic pathway for the formation of the ether bond in halophiles is still unknown. Acidophilic thermophiles contain tetraalkyl glycerolipids with their two glycerol moieties linked across their membranes, which prevents them from being freeze-fractured.

Many anaerobic bacteria are highly enriched in plasmalogens. For example, *Clostrid-ium butyricum* contains significant amounts of ethanolamine plasmalogens and *Megas-phaera elsdenii* has been reported to contain very large quantities of plasmenyl ethanolamine and plasmenylserine. However, despite the large pool of plasmalogens in such anaerobes, no information has emerged about how they synthesize the alk-1-enyl ether bond.

4. Physical properties

Replacement of ester linkages in glycerolipids with ether bonds mainly affects hydrophobic-hydrophilic interactions. Nevertheless, the closer linear packing arrangement attainable with ether-linked moieties also is capable of influencing the polar head group region of phospholipids. The novel placement of the $\Delta 1$ double bond in plasmalogens can also exert effects on stereochemical relationships and therefore, the presence of an ether-linkage in phospholipids can modify both the configuration and functional properties of membranes.

In model membranes, ether-linked lipids have been shown to decrease ion permeability, surface potential, and lower the phase temperature of membrane bilayers when compared to their diacyl counterparts. *Clostridium butyricum* appears to be able to regulate the stability of the bilayer arrangement of membranes by altering the ratio of ether versus acyl type of ethanolamine phospholipids in response to changes in the degree of lipid unsaturation of the membranes. The experiments with bacteria indicate that the substitution of plasmenylethanolamine for phosphatidylethanolamine in biomembranes would have only small effects on lipid melting transitions, whereas the tendency to form non-lamellar lipid structures would be significantly increased.

5. Biologically active ether lipids

5.1. Platelet-activating factor

In 1979, the chemical structure of PAF was identified as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (Fig. 2). The semisynthetic preparation tested in these initial experiments caused aggregation of platelets at concentrations as low as 10^{-11} M and induced an antihypertensive response when as little as 60 ng were administered intravenously to hypertensive or normotensive rats. It is now known that PAF, a phospholipid secreted by numerous cells, exerts many different types of biological responses (Table 1) and it has been implicated as a contributing factor in the pathogenesis of such diverse disease processes as asthma, hypertension, allergies, inflammation, and anaphylaxis, to name only a few.

PAF has been isolated and very well characterized from a number of cellular sources. Basophils, neutrophils, platelets, macrophages, monocytes and mast, endothelial, and HL-60 cells are among the highest producers of PAF when stimulated by agonists such as chemotactic peptides, zymosan, thrombin, calcium ionophores, antigens, bradykinin,

I. In vivo responses:

- 1. Bronchoconstriction \uparrow
- 2. Systemic blood pressure \downarrow
- 3. Pulmonary resistance ↑
- 4. Dynamic lung compliance \downarrow
- 5. Pulmonary hypertension and edema ↑
- 6. Heart rate ↑
- 7. Hypersensitivity responses ↑
- 8. Vascular permeability ↑

II. Cellular responses:

- 1. Aggregation of neutrophils and platelets \uparrow
- 2. Degranulation of platelets, neutrophils, and mast cells \uparrow
- 3. Shape changes in platelets, neutrophils, and endothelial cells \uparrow
- 4. Chemotaxis and chemokinesis in neutrophils \uparrow

III. Biochemical responses:

- 1. Ca^{2+} uptake \uparrow
- 2. Respiratory burst and superoxide production \uparrow
- 3. Protein phosphorylation ↑
- 4. Arachidonate turnover ↑
- 5. Phosphoinositide turnover ↑
- Protein kinase ↑
 - protein kinase C
 - mitogen-activated protein kinase
 - G-protein receptor kinases
 - protein tyrosine kinase
- 7. Glycogenolysis ↑
- 8. Tumor necrosis factor production \uparrow
- 9. Interleukin 2 production \downarrow
- 10. Activation of immediate-early genes, e.g., c-fos and c-jun, zif/268 ↑

ATP, C_{5a} , collagen, and disease states. The amount of PAF produced by various stimuli is dependent on the cell type and the specific agonist used. Most animal tissues also have the capacity to produce PAF by de novo synthesis (see Section 6.3.2).

Other acetylated glycerolipids that are structurally related to PAF include 1-alkyl-2acetyl-*sn*-glycerols and the plasmalogen and acyl analogs of PAF that possess choline or ethanolamine moieties (Fig. 2). Both the alkylacetylglycerols (perhaps via phosphorylation) and the choline plasmalogen analog of PAF can mimic the actions of PAF, perhaps through their interactions with the PAF receptor. Biological potencies of the PAF analogs range from 5- to 4000-fold weaker than PAF [18].

An unnatural chemically synthesized analog of PAF, 1-alkyl-2-methoxy-*sn*-glycero-3-phosphocholine (Fig. 2) and related derivatives, possesses unique highly selective antitumor activity [19]. Clinical studies in Europe have shown a promising therapeutic potential for the methoxy analog in treating certain types of human cancers. Although its mode of action has been difficult to ascertain, the primary site of action of these PAF analogs is the plasma membrane rather than the cell nucleus. The cytotoxic activity of this antineoplastic phospholipid is apparently due to its ability to prevent the formation of membranes by blocking phosphatidylcholine synthesis (Chapter 8) via the inhibition of CTP:phosphocholine cytidylyltransferase, the rate-limiting enzyme in phosphatidylcholine biosynthesis [20].

5.2. Other ether-linked mediators

In addition to PAF and eicosanoids, 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine yields 1-alkyl-2-acyl-*sn*-glycero-3-phosphate when acted upon by phospholipase D [21]. Both the alkylacyl- and diacylglycerols share in their ability to increase responses (priming) of neutrophils to other stimuli of arachidonic acid release and the oxidative burst. However, only the diacylglycerol primes for the formation of lipoxygenase products.

5.3. Oxidized phospholipids

Oxidation of the phospholipids of plasma lipoproteins generates bioactive phospholipid species with PAF-like activity [13]. A complex mixture of oxidation products is formed but the species that bind and act through the PAF receptor are alkyl ether-linked and contain short-chain oxidized residues in the sn-2 position derived from polyunsaturated acyl chains. Normally, PAF analogs containing sn-2 chains longer than four carbons have little activity but the introduction of an oxidized group at the end of the chain yields longer-chain active analogs. The PAF acetylhydrolase (Section 7.3.1) can remove the oxidized chains to inactivate these products. These oxidized species may play an important role in inflammation and development of atherosclerotic plaques and other cardiovascular disorders.

5.4. Receptors, overexpression, and knockout mice

The cDNA encoding the cell surface PAF receptor has been cloned and the primary structure sequenced from a number of cells/tissues including guinea pig lung, human neutrophils, HL-60 cells (granulocytic form), and human heart [13,14]. Human and guinea pig receptors consist of 342 amino acids with a C-terminal cytoplasmic tail possessing serine and threonine residues which could be potential sites for regulation via phosphorylation. The PAF receptor is typical of other members of the family of G-protein-coupled receptors, which span the membrane seven times (e.g., rhodopsin, β_1 and β_2 adrenergic, D₂-dopamine, and M1–M5 muscarinic). Based on modeling and site-directed mutagenesis of the receptor it is proposed that the central portion of the receptor and the histidine residues 188, 248, and 249 form the PAF binding pocket. It is also deemed likely that there is a disulfide bond between the cysteine residues at positions 90 and 163. A mutation in the third transmembrane domain resulted in a constitutively active receptor. It has been shown in other studies that the third intracellular loop is necessary for initiating phosphatidylinositol turnover. The fate of the receptor-bound PAF is unknown.

The role of PAF in vivo has been examined by overexpressing the guinea pig receptor in mice [8,14]. These mice had an increased death rate in response to

endotoxin and surprisingly developed melanocytic tumors of the skin. Ishii and Shimizu [14] have also generated PAF receptor knockout mice. These mice had less severe anaphylactic responses including diminished cardiovascular instability, alveolar edema, and airway constriction than did wild-type mice. Unexpectedly, the receptor-deficient animals reproduced normally and remained susceptible to endotoxin. The existence of a second PAF receptor might explain these results. Further studies are required to resolve the somewhat contradictory observations. In humans lacking PAF acetylhydrolase, an enzyme that degrades PAF, an increased severity of asthma, coronary artery disease and stroke was observed. Several review articles [7,8,13,14] discuss the role of PAF receptors in signal transduction, cloning, sequencing, and related studies.

5.5. Receptor antagonists

A number of PAF antagonists are available that block binding of PAF to its receptor. Some of these antagonists are derived from plants, such as *Ginkgo biloba*, while others are structural analogs of PAF, and yet others are chemically synthesized compounds found through screening. Although some of the inhibitors effectively block PAF responses in certain systems, they have not proven highly effective as anti-inflammatory drugs. It is possible that the drugs do not gain access to all PAF receptors in vivo, or that the network of inflammatory mediators synergizes to overcome suppression of the PAF receptor.

6. Enzymes involved in ether lipid synthesis

In view of the vast literature about the enzymes involved in the metabolism of etherlinked lipids and PAF, the reader should consult the various reviews on this subject [5,10-13].

6.1. Ether lipid precursors

6.1.1. Acyl Co-A reductase

Fatty alcohol precursors of ether lipids are derived from acyl-CoAs via a fatty aldehyde intermediate in a reaction sequence catalyzed by a membrane-associated acyl-CoA reductase (Fig. 3A). A cytosolic form of the reductase from bovine heart has also been described.

Acyl-CoA reductases associated with membrane systems use acyl-CoA substrates, and in mammalian cells, they exhibit a specific requirement for NADPH. The fatty alcohols produced by the reductase can be oxidized back to the fatty acids by microsomes in the presence of NAD.

Although only traces of fatty aldehydes can normally be detected as an intermediate in these reactions, the use of trapping agents such as semicarbazide has documented that aldehydes are indeed formed as intermediates. Acyl-CoA reductase prefers saturated substrates over acyl-CoAs that are unsaturated; in fact, the enzyme in brain microsomes is not able to convert polyunsaturated moieties to fatty alcohols. Some evidence



Fig. 3. Enzymatic synthesis (A) and oxidation (B) of long-chain fatty alcohols by (I) acyl-CoA reductase and (II) fatty alcohol oxidoreductase, respectively.

indicates that, at least in brain, acyl-CoA reductase is localized in microperoxisomes. Topographical studies of microperoxisomal particles have revealed the acyl-CoA reductase activity is located at the cytosolic surface of these membranes. In rabbit Harderian glands and *Euglena gracilis*, the NADPH-dependent reductase appears to be closely coupled with fatty acid synthase and it has been suggested that the fatty acid bound to acyl carrier protein, rather than acyl-CoA, is the substrate for this reductase.

6.1.2. Dihydroxyacetone-P acyltransferase

Presumably, dihydroxyacetone-P acyltransferase is present in all mammalian cells that synthesize alkylglycerolipids, since the acylation of dihydroxyacetone-P is an obligatory step in the biosynthesis of the ether bond in glycerolipids. On the other hand, the quantitative importance of the pathways utilizing dihydroxyacetone-P versus *sn*-glycerol-3-P in the biosynthesis of glycerolipid esters (Chapter 8) has never been firmly established in intact cells.

Current evidence indicates that dihydroxyacetone-P acyltransferase, as well as alkyldihydroxyacetone-P synthase, is localized in microperoxisomes. Nevertheless, many studies of these enzymes have been done with microsomal and/or mitochondrial preparations; however, it is well known that microperoxisomes sediment with microsomes and large peroxisomes sediment with mitochondria under the usual preparation conditions for these subcellular fractions. Investigations of the topographical orientation of dihydroxyacetone-P acyltransferase in membrane preparations from rabbit Harderian glands and rat brains indicate that unlike most other enzymes in glycerolipid metabolism, dihydroxyacetone-P acyltransferase appears to be located on the internal side of microsomal vesicles.

6.2. Ether lipids

A)

6.2.1. O-Alkyl bond: mechanism of formation

Formation of the alkyl ether bond in glycerolipids is catalyzed by alkyldihydroxyacetone-P synthase (Fig. 4). This reaction, which forms alkyldihydroxyacetone-P as the first detectable intermediate in the biosynthetic pathway for ether-linked glycerolipids, is





(alkyldihydroxyacetone-P)

Fig. 4. The reaction that forms the *O*-alkyl bond is catalyzed by (I) alkyldihydroxy acetone-P synthase and is thought to proceed via a ping-pong mechanism. The abbreviation DHAP in this illustration designates dihydroxyacetone-P. Upon binding of acyl-DHAP to the enzyme, alkyl-DHAP synthase, the pro-*R* hydrogen at carbon atom 1 is exchanged by an enolization of the ketone, followed by release of the acyl moiety to form an activated enzyme–DHAP complex. The carbon atom at the 1 position of DHAP in the enzyme complex is thought to carry a positive charge that may be stabilized by an essential sulfhydryl group of the enzyme; thus, the incoming alkoxide ion reacts with the carbon 1 atom to form the ether bond of alkyl-DHAP. It has been proposed that a nucleophilic cofactor at the active site covalently binds the DHAP portion of the substrate.

unique in mammals since it is the only one known where a fatty alcohol can be directly substituted for a covalently linked acyl moiety. Alkyldihydroxyacetone-P synthase has been primarily investigated in microsomal preparations; however, as with dihydroxyacetone-P acyltransferase, there is general agreement that the synthase activity is peroxisomal. The alkyl synthase cDNAs from guinea pig and human liver reveal that both proteins contain a peroxisomal targeting signal 2 [22]. The importance of peroxisomes in ether lipid synthesis has been highlighted by the finding that patients with Zellweger syndrome (lacking peroxisomes) and related peroxisomal-deficient diseases have extremely low levels of plasmalogens and ether lipids.

Kinetic experiments with a partially purified enzyme from Ehrlich ascites cells have suggested the reaction catalyzed by alkyldihydroxyacetone-P synthase involves a ping-pong rather than sequential-type mechanism, with an activated enzyme–dihydroxyacetone-P intermediary complex playing a central role. The existence of this intermediate would explain the reversibility of the reaction, since the enzyme–dihydroxyacetone-P complex can react with either fatty alcohols (forward reaction) or fatty acids (back reaction). This unusual enzymatic mechanism is also consistent with other known properties of alkyldihydroxyacetone-P synthase. Acyldihydroxyacetone-P acylhydrolase does not appear to participate in this mechanism since its activity is not present in the purified synthase preparation.

A number of novel features characterize the reaction that forms alkyldihydroxyacetone-P. The pro-R hydrogen at C-1 of the dihydroxyacetone-P moiety of acyldihydroxyacetone-P exchanges with water, without any change in the configuration of the C-1 carbon. Cleavage of the acyl group of acyldihydroxyacetone-P occurs before the addition of the fatty alcohol, and either fatty acids or fatty alcohols can bind to the activated enzyme-dihydroxyacetone-P complex to produce acyldihydroxyacetone-P or alkyldihydroxyacetone-P, respectively. There is no evidence for a Schiff's base being formed. Nevertheless, a ketone function is an essential feature of the substrate, acyldihydroxyacetone-P. In addition, mass spectrometric analyses have clearly shown that the oxygen of the ether bond is donated by the fatty alcohol and both oxygens of the acyl linkage of acyldihydroxyacetone-P are recovered in the fatty acid released.

The cDNAs for human and guinea pig alkyldihydroxyacetone-P synthase have been cloned and expressed. The apparent molecular mass of the enzyme from guinea pig is 65 kDa on polyacrylamide gel electrophoresis. The mature enzymes of both human and guinea pig have a predicted mass of 67 kDa. The enzyme is synthesized as a 658 amino acid precursor containing an N-terminal presequence of 58 amino acids encoding the peroxisomal targeting signal 2 motif, which is removed in the mature protein. In studies of the structure and mechanism of action of the enzyme, de Vet et al. [22] made the surprising finding that the enzyme contains a FAD binding domain. They demonstrated the presence of FAD in the enzyme and found that the FAD cofactor is required for activity of the enzyme. The FAD participates directly in catalysis and becomes reduced upon incubation with acyldihydroxyacetone-P. This finding suggests that the dihydroxyacetone-P moiety has been oxidized as the acyl chain is removed. Evidence indicated that the unidentified oxidized intermediate is not covalently linked to the enzyme but can be washed off the enzyme. Addition of fatty alcohol and synthesis of alkyldihydroxyacetone-P results in reoxidation of the FADH₂. Normally, acylhydrolase reactions proceed by acyl oxygen fission in which only one of the oxygens of the ester bond remains with the acyl chain; alkyl oxygen fission, where both oxygens of the ester bond remain with the released acyl chain, as catalyzed by the alkyl synthase, is very unusual. The proposed oxidized dihydroxyacetone-P intermediate is yet to be identified. These new findings and available systems may soon reveal the exact mechanism by which this exciting enzyme is able to synthesize an ether bond.

Alkyldihydroxyacetone-P synthase exhibits a very broad specificity for fatty alcohols of different carbon chain lengths. On the other hand, the specificity of the synthase for acyldihydroxyacetone-P possessing different acyl chains is less well understood, primarily because of their lack of availability.

6.2.2. O-Alkyl analog of phosphatidic acid and alkylacylglycerols

Once alkyldihydroxyacetone-P is synthesized, it can be readily converted to the *O*-alkyl analog of phosphatidic acid (Fig. 5) in a two-step reaction sequence. The NADPH-dependent oxidoreductase, located on the cytosolic side of peroxisomal membranes, is capable of reducing the ketone group of both the alkyl and acyl analogs of dihydroxyacetone-P. Dietary ether lipids can also enter this pathway, since alkylglycerols formed via the catabolism of dietary ether-linked lipids during absorption are known to be phosphorylated by an ATP:alkylglycerol phosphotransferase to form 1-alkyl-2-lyso-*sn*-glycerol-3-P (Fig. 5, Reaction IV), which can then be acylated by an acyl-CoA acyltransferase to produce plasmanic acid, the *O*-alkyl analog of phosphatidic acid. The latter can be dephosphorylated to alkylacylglycerols which occupy an important branch point in the ether lipid pathway in a manner analogous to the diacylglycerols. Reaction steps beginning with 1-alkyl-2-acyl-*sn*-glycerol in the routes leading to the more complex ether-linked neutral lipids and phospholipids (Fig. 5, Reactions V, VI, and VII) are thought to be catalyzed by the same enzymes as those involved in the



Fig. 5. Biosynthesis of membrane phospholipids from alkyldihydroxyacetone-P, the first detectable intermediate formed in the biosynthetic pathway for ether-linked glycerolipids. Enzymes responsible for catalyzing the reactions shown are (I) NADPH:alkyldihydroxyacetone-P oxidoreductase, (II) acyl-CoA:1-alkyl-2-lyso*sn*-glycero-3-P acyltransferase, (III) 1-alkyl-2-acyl-*sn*-glycero-3-P phosphohydrolase, (IV) ATP:1-alkyl-*sn*glycerol phosphotransferase, (V) CDP-choline:1-alkyl-2-acyl-*sn*-glycerol cholinephosphotransferase, (VI) CDP-ethanolamine:1-alkyl-2-acyl-*sn*-glycerol ethanolaminephosphotransferase, and (VII) acyl-CoA:1-alkyl-2-acyl-*sn*-glycerol acyltransferase.

pathways originally established by Kennedy and co-workers in the late 1950s for the diacylglycerolipids (Chapter 8).

6.2.3. Neutral ether-linked glycerolipid

Alkyldiacylglycerols, the *O*-alkyl analog of triacylglycerols, are produced by acylation of 1-alkyl-2-acyl-*sn*-glycerols in a reaction catalyzed by an acyl-CoA acyltransferase (Fig. 5, Reaction VII). The acyltransferase can also acylate 1-alk-1-enyl-2-acyl-*sn*-glycerols to form the 'neutral plasmalogen' analog of triacylglycerols. In addition, an acetylated *O*-alkyl analog of triacylglycerols has been shown to be synthesized from 1-alkyl-2-acetyl-*sn*-glycerols in HL-60 cells. The biological function of these ether-linked neutral lipids is unknown at the present time.

6.2.4. O-Alkyl choline- and ethanolamine-containing phospholipids

1-Alkyl-2-acyl-*sn*-glycerols, derived from the alkyl analog of phosphatidic acid by the action of a phosphohydrolase, also are utilized as substrates by cholinephosphotransferase (Fig. 5, Reaction V) and ethanolaminephosphotransferase (Fig. 5, Reaction VI) to form plasmanylcholines and plasmanylethanolamines, the alkyl analogs of phosphatidylcholine and phosphatidylethanolamine. Plasmanylcholine is the membrane source of lyso-PAF, the ether lipid precursor of the potent biologically active phospholipid, PAF, whereas plasmanylethanolamine is the direct precursor of ethanolamine plasmalogens.



Fig. 6. Biosynthesis of ethanolamine plasmalogens by $\Delta 1$ -alkyl desaturase. Components of the enzyme complex responsible for this unusual type of desaturation between carbons 1 and 2 of the *O*-alkyl chain are (I) NADH cytochrome b_5 reductase, (II) cytochrome b_5 , and (III) $\Delta 1$ -alkyl desaturase. GPE, glycerophosphoethanolamine.

6.2.5. Ethanolamine plasmalogens

The Δ 1-alkyl desaturase, a microsomal mixed-function oxidase system, is responsible for the biosynthesis of ethanolamine plasmalogens from alkyl lipids (Fig. 6). The alkyl desaturase, which produces the alk-1-enyl grouping, is a unique enzyme, since it can specifically and stereospecifically abstract hydrogen atoms from C-1 and C-2 of the *O*-alkyl chain of an intact phospholipid molecule, 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, to form the *cis* double bond of the *O*-alk-1-enyl moiety. Only intact 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine is known to serve as a substrate for the alkyl desaturase.

Like the acyl-CoA desaturases (Chapter 7), the Δ 1-alkyl desaturase exhibits the typical requirements of a microsomal mixed-function oxidase: molecular oxygen, a reduced pyridine nucleotide, cytochrome b_5 , cytochrome b_5 reductase, and a terminal desaturase protein that is sensitive to cyanide. The precise reaction mechanism responsible for the biosynthesis of the ethanolamine plasmalogens is unknown. However, it is clear from an investigation with a tritiated fatty alcohol, that only the 1S and 2S (*erythro*) labeled hydrogens are lost during the formation of the alk-1-enyl moiety of ethanolamine plasmalogens.

6.2.6. Choline plasmalogens

 Δ 1-Alkyl desaturase does not utilize 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine as a substrate. In fact, biosynthesis of the significant quantities of choline plasmalogens that occurs in some heart tissues remains an enigma, although most available data strongly imply that they are derived from the ethanolamine plasmalogens. Considerable evidence has accumulated to indicate that a combination of phospholipase A₂, lysophospholipase D, acyltransferase, phosphohydrolase, and cholinephosphotransferase activities participate in the conversion of plasmenylethanolamine to plasmenylcholine. Direct base exchange, coupled phospholipase C/cholinephosphotransferase reactions, and the methylation of the ethanolamine moiety could also contribute to the synthesis of plasmenylcholine [23,24]. Available evidence indicates that direct polar head group remodeling mechanisms (Fig. 7) or a combined enzymatic modification of the *sn*-2 and *sn*-3 positions of ethanolamine plasmalogens (Fig. 8) best explain how choline plasmalogens are formed.



Fig. 7. Possible pathways for biosynthesis of choline plasmalogens via the modification of the *sn*-3 polar head group of ethanolamine plasmalogens are catalyzed directly by (I) a base exchange enzyme or (II) *N*-methyltransferase. A combination of other enzymatic reactions can also result in the replacement of the ethanolamine moiety of plasmenylethanolamine to produce plasmenylcholines; the enzymes responsible include (III) phospholipase C, (IV) the reverse reaction of ethanolamine phosphotransferase, (V) phospholipase D, (VI) a phosphohydrolase, and (VII) cholinephosphotransferase. Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; Etn, ethanolamine; GPE, glycerophosphoethanolamine.

6.3. PAF and related bioactive species

6.3.1. Remodeling route

The remodeling pathway of PAF synthesis (Fig. 9) is thought to be the primary contributor to hypersensitivity reactions and for this reason this route has been implicated in most pathological responses involving PAF. Biosynthesis of PAF during inflammatory cellular responses or following various agonist stimulation occurs via the enzymatic remodeling of membrane-bound alkylacylglycerophosphocholines by replacing an acyl moiety with an acetate group. The enzymes responsible for catalyzing the hydrolytic deacylation step appear to be highly specific for the molecular species of alkylacylglycerophosphocholines possessing an arachidonoyl moiety at the sn-2 position. The initial reaction that produces lyso-PAF requires either the combined actions of a membrane-associated CoA-*independent* transacylase/phospholipase A₂ (Fig. 9, Reaction II; indirect route) or can be catalyzed in a single direct hydrolytic step by a phospholipase A₂ (Fig. 9, Reaction I). A CoA-*dependent* transacylase (reversal of an acyl-CoA acyltransferase



Fig. 8. Biosynthesis of plasmenylcholine via the modification of both the sn-2 acyl and sn-3 polar head group moieties of plasmenylethanolamine. (I) phospholipase A₂, (II) CoA-independent transacylase, (III) lysophospholipase C, (IV) lysophospholipase D, (V) a phosphotransferase, (VI) acyl-CoA acyltransferase, (VII) phosphohydrolase, and/or (VIII) cholinephosphotransferase. Abbreviations: Etn, ethanolamine; Cho, choline; GPE, sn-glycero-3-phosphocholine.



Fig. 9. Biosynthesis of PAF via the remodeling pathway. Lyso-PAF, the immediate precursor of PAF, can be formed from 1-alkyl-2-acyl-*sn*-3-glycerophosphocholine through the direct action of (1) a phospholipase A_2 or (II) a CoA-*independent* transacylase. The lysoplasmenylethanolamine (or other potential ethanolamineor choline-containing lysoglycerophospholipids) is thought to be generated by (III) a phospholipase A_2 that exhibits a high degree of selectivity for substrates having an arachidonoyl moiety at the *sn*-2 position. The transacylase (II) appears to possess both acyl transfer and phospholipase A_2 hydrolytic activities, which could exist as a single protein or as a tightly associated complex of two distinctly different proteins. The lyso-PAF produced by either the transacylation (II) or direct phospholipase A_2 (I) reactions can then be acetylated to form PAF by (IV) an acetyl-CoA acetyltransferase.

reaction) is also capable of generating lyso-PAF (Fig. 10, Reaction I). Two reviews have focused on the different types of transacylase reactions involved in the remodeling of phospholipids [25,26].

A number of studies indicate that the 85 kDa cytosolic phospholipase A_2 is likely the phospholipase responsible for release of arachidonic acid and PAF synthesis in stimulated cells. It is highly selective for arachidonate as is the CoA-independent transacylase. One of the most convincing findings showing that this enzyme is responsible for initiating the remodeling pathway is that macrophages from cytosolic phospholipase A_2 knockout mice almost completely lose their ability to synthesize both PAF and eicosanoids [13,14]. Since the cytosolic phospholipase A_2 does not distinguish between the ester and ether linkage in the *sn*-1 position, acetylated products recovered from cells reflect the composition of the choline-containing phosphoglycerides. The activity is regulated by phosphorylation of the enzyme and by translocation from the cytosol to membranes, which requires concentrations of $\mu M Ca^{2+}$; Ca²⁺ is not required for the hydrolytic mechanism of cytosolic phospholipase A_2 .

The acetyltransferase responsible for the final step in the synthesis of PAF (Fig. 9,



Fig. 10. Involvement of a CoA-*dependent* transacylase in the production of lyso-PAF for the synthesis of PAF and the remodeling of the sn - 2 acyl group of membrane phospholipids. The enzymes responsible for catalyzing these reactions are (I) the CoA-*dependent* transacylase (with CoA as the acyl acceptor), (II) acetyl CoA:lyso-PAF acetyltransferase, and (III) an acyl-CoA:lysophospholipid acyltransferase. The reaction depicted for the CoA-dependent transacylase represents the reversal of the reaction catalyzed by acyl-CoA:lysophospholipid acyltransferase. GPE designates sn-3-glycerophosphotethanolamine.

Reaction IV) is membrane-bound and, like the CoA-independent transacylase, has neither been purified nor its cDNA cloned. This membrane-bound enzyme can also acetylate both the alk-1-enyl and acyl analogs of lyso-PAF and utilizes short-chain acyl-CoAs ranging from C_2 to C_6 as substrates. Several studies indicate that the enzyme is activated by phosphorylation even though the unphosphorylated enzyme appears to have a basal activity. In studies of human neutrophils, Nixon et al. [27] have concluded from studies with MAP kinase inhibitors and recombinant cytosolic phospholipase A_2 and MAP kinases that the acetyl-CoA:lyso-PAF acetyltransferase is specifically activated by the p38 stress-activated MAP kinase but not by p42 and p44 ERKs. In contrast, cytosolic phospholipase A_2 is activated in the cells by both the ERKs and p38 kinase. Related findings suggest that the production of phosphatidic acid by phospholipase D specifically activates the p38 kinase cascade but not the ERKs. Identification of the specific protein kinases responsible for the direct activation of cytosolic phospholipase A_2 and the acetyltransferase in intact cells is complicated by cross-talk between the kinases, including protein kinase C.

Regulation of the CoA-independent transacylase activity (Fig. 9, Reaction II) is poorly understood. It has been demonstrated that production of lyso-PAF via the transacylation step can occur in either a CoA-*independent* (Fig. 9) or CoA-*dependent* (Fig. 10) manner [20,21]. With the CoA-*independent* transacylase, ethanolamine lysoplasmalogens as well as other ethanolamine- or choline-containing lysoglycerophosphatides serve as the acyl acceptor for the selective transfer of an arachidonoyl moiety from alkylacylglycerophosphocholine. CoA itself, instead of a lysophospholipid, is the acyl acceptor in the reaction catalyzed by the CoA-dependent transacylase (Fig. 10). This type of transacylation is thought to represent the reverse reaction of that catalyzed by an acyl-CoA:lyso-PAF acyltransferase but is not selective for arachidonate. In addition to participating in the formation of lyso-PAF, the transacylases also serve an important role in the remodeling of acyl moieties located at the *sn*-2 position of the choline- and ethanolamine-containing phospholipids.

The lysoplasmalogen or other lysophospholipid acceptors that are substrates for the transacylases appear to be formed by the direct action of a phospholipase A_2 on the appropriate membrane-associated phospholipid which simultaneously releases arachidonic acid for its subsequent metabolism to bioactive eicosanoid products (Chapter 13). In both the direct and indirect routes of lyso-PAF production, the action of a phospholipase A_2 is required; it is plausible that both routes participate in PAF synthesis to varying degrees depending on conditions. Since both eicosanoid and PAF mediators can be formed via the remodeling pathway and these mediators can act synergistically, the assessment of biological responses following cell activation can often be difficult to interpret.

6.3.2. De novo route

PAF biosynthesis via the de novo pathway [10,11] is thought to be the primary source of the physiological levels of PAF in cells and blood (Fig. 11). Both fatty acids and neurotransmitters can stimulate the de novo synthesis of PAF. The sequence of enzymatic reactions (Fig. 11) involved in the de novo route include (a) acetylation of 1-alkyl-2-lyso-sn-glycero-3-P by an acetyl-CoA-dependent acetyltransferase (Reaction I), (b) dephosphorylation of 1-alkyl-2-acetyl-sn-glycero-3-P (Reaction II), and (c) the transfer of phosphocholine from CDP-choline to 1-alkyl-2-acetyl-sn-glycerol by a dithiothreitol-insensitive cholinephosphotransferase (Reaction III) to form PAF. The acetyltransferases associated with the remodeling (Fig. 9) and de novo routes (Fig. 11) possess distinctly different properties and substrate specificities. Also, the dithiothreitolinsensitivity of this cholinephosphotransferase contrasts with the inhibitory effect of dithiothreitol on the cholinephosphotransferase that synthesizes phosphatidylcholine and plasmanylcholine from diacylglycerols and alkylacylglycerols, respectively. In addition, the two dissimilar cholinephosphotransferase activities that synthesize PAF and phosphatidylcholine exhibit different pH optima and respond differently to detergents, ethanol, temperature, and substrates. Although the enzymes in the de novo pathway exhibit a relatively high degree of substrate specificity, the sn-1 acyl analogs of the corresponding O-alkyl equivalents can also be utilized as substrates by the acetyltransferase, phosphohydrolase, and the dithiothreitol-insensitive cholinephosphotransferase.

6.3.3. PAF transacetylase

Two novel CoA-independent transacetylases that use PAF as the donor molecule (Fig. 12) are PAF: lysophospholipid transacetylase and PAF: sphingosine transacetylase [28]. Both transacetylases have no requirement for Ca^{2+} , Mg^{2+} , or CoA. The PAF: lysophospholipid transacetylase transfers the acetyl group from PAF to a variety



Fig. 11. Biosynthesis of PAF via the de novo pathway. The three-step reaction sequence in this route, beginning with 1-alkyl-2-lyso-*sn*-glycero-3-P as the precursor, is catalyzed by (I) acetyl-CoA:alkyllysoglycero-P acetyltransferase, (II) alkylacetylglycero-P phosphohydrolase, and (III) CDP-choline:alkylacetylglycerol cholinephosphotransferase.

of lysophospholipids to form a series of PAF analogs. Among all the lysophospholipids tested, acyllysoglycerophosphocholine is the most active acetyl group acceptor. In addition, *cis*-9-octadecen-1-ol can also serve as acetate acceptor, whereas alkylglycerol, acylglycerol, or cholesterol are inactive. Biochemical studies suggest that the CoA-independent transacetylase differs from the CoA-independent transacylase that transfers long-chain acyl moieties.



Fig. 12. PAF transacetylase transfers the acetate moiety of PAF to other selective substrates to produce a plasmalogen analog (I) and acetylsphingosine (II). GPE, glycerophosphoethanolamine.

The PAF : sphingosine transacetylase transfers the acetate group from PAF to sphingosine forming *N*-acetylsphingosine (C_2 -ceramide). The enzyme has a narrow substrate specificity and strict stereochemical configuration requirement. Ceramide, sphingosylphosphocholine, stearylamine, sphingosine-1-phosphate, or sphingomyelin are not substrates, whereas sphinganine has a limited capacity to accept the acetate from PAF. Only the naturally synthesized *D-erythro* isomer but not the synthetic *L-erythro-*, *D-threo-*, or *L-threo*-isomer of sphingosine can serve as a substrate. Both PAF : lysophospholipid transacetylase and PAF : sphingosine transacetylase have similar tissue distributions. The PAF : sphingosine transacetylase is located in mitochondria, microsomes, and cytosol with mitochondria having the highest specific activity. Physiological levels of C_2 ceramide (in μ M range) have been detected in both undifferentiated and differentiated HL-60 cells.

Rat kidney transacetylases from mitochondria/microsomes and cytosols have an apparent molecular mass of 40 kDa. Both purified enzymes from membranes and cytosols contain three catalytic activities; PAF: lysophospholipid transacetylase, PAF: sphingosine transacetylase, and PAF acetylhydrolase (PAF-AH). A search using a protein sequence data bank indicates that these sequences have homology with the sequences present in bovine PAF-AH II (Section 7.3.1).

The substrate specificity, kinetic parameters, and inhibitor effects suggest that the three individual catalytic activities of the transacetylase have different dependencies on the thiol-containing residue(s) of the enzyme, i.e., cysteine. Furthermore, the non-responsiveness of the purified cytosolic transacetylase to phosphatidylserine activation indicates that membrane and cytosolic transacetylase may be posttranslationally distinct.

Analysis of a series of site-directed mutant PAF-AH II proteins in CHO-K1 cells shows that lysophospholipid transacetylase is decreased, whereas PAF-AH activity is not affected in C120S and G2A mutants. Thus, Cys¹²⁰ and Gly² are implicated in the catalysis of the lysophospholipid transacetylase reaction in this enzyme. It appears that *N*-myristoylation is not required for PAF-AH activity.

Several lines of evidence indicate that transacetylase activity has a physiological role in vivo. With intact differentiated HL-60 cells, $[^{3}H]$ acetate from $[^{3}H]$ PAF can be incorporated into alk-1-enyl $[^{3}H]$ acetylglycerophosphoethanolamine in the presence of ionophore A23187, but not in its absence. In endothelial cells stimulated by ATP, bradykinin, and ionophore A23187, acylacetylglycerophosphocholine is the predominant product and the radiolabelled acetate group of PAF is incorporated into acylacetylglycerophosphocholine in a time-dependent fashion.

In ATP-activated cells, PAF: acyllysoglycerophosphocholine transacetylase and formation of acylacetylglycerophosphocholine are concurrently and transiently induced, while PAF: sphingosine transacetylase and PAF-AH activities remain unchanged. Evidence indicates that tyrosine protein kinase and protein kinase C are directly or indirectly involved in the activation of the transacetylase activity through protein phosphorylation. In addition, ATP induces the translocation of acyllysoglycerophosphocholine transacetylase from cytosol to membranes and also increases the specific enzyme activity on the membrane. Collectively, the three catalytic activities of the transacetylase are regulated in agonist-activated cells through posttranslational modifications (such as reversible phosphorylation/dephosphorylation, myristoylation, etc.) and translocation of the enzyme from cytosol to membranes.

7. Catabolic enzymes

7.1. Ether lipid precursors

7.1.1. Fatty alcohols

Fatty alcohols are oxidized to fatty acids via a fatty alcohol:NAD⁺ oxidoreductase, a microsomal enzyme found in most mammalian cells (Fig. 3B). The high activity of this enzyme probably accounts for the extremely low levels of unesterified fatty alcohols generally found in tissues or blood. Detection of fatty aldehydes, by trapping them as semicarbazide derivatives during oxidation of the alcohol, suggests that the fatty alcohol oxidoreductase catalyzes a two-step reaction that involves an aldehyde intermediate.

7.1.2. Dihydroxyacetone-P and acyldihydroxyacetone-P

Dihydroxyacetone-P can be diverted from its precursor role in ether lipid synthesis when it is converted to *sn*-glycerol-3-P by glycerol-3-P dehydrogenase. Another bypass that prevents the formation of alkyldihydroxyacetone-P occurs if the ketone function of acyldihydroxyacetone-P is first reduced by an NADPH-dependent oxidoreductase, since the product, 1-acyl-2-lyso-*sn*-glycerol-3-P, can then be converted to different diacyl types of glycerolipids. Obviously, the metabolic removal and/or formation of fatty



Fig. 13. Cleavage of the O-alkyl linkage in glycerolipids (A) is catalyzed by (I) tetrahydropteridine (Pte-H₄)-dependent alkyl monooxygenase. The fatty aldehyde product can be either reduced to a longchain fatty alcohol by (II) a reductase or oxidized to a fatty acid by (III) an oxidoreductase. Removal of the O-alk-1-enyl moiety from plasmalogens (B) is catalyzed by a plasmalogenase. As with the O-alkyl monooxygenase, the fatty aldehyde can be converted either to the corresponding fatty alcohol or fatty acid. GPE, glycerophosphoethanolamine.

alcohols, dihydroxyacetone-P, or acyldihydroxyacetone-P from the ether lipid precursor pool represent important control points for regulating the ether lipid pathway.

7.2. Ether-linked lipids

7.2.1. O-Alkyl cleavage enzyme

Oxidative cleavage of the *O*-alkyl linkage in glycerolipids is catalyzed by a microsomal tetrahydropteridine (Pte \cdot H₄)-dependent alkyl monooxygenase (Fig. 13A). The required cofactor, Pte \cdot H₄, is regenerated from the Pte \cdot H₂ by an NADPH-linked pteridine reductase, a cytosolic enzyme. Oxidative attack on the ether-linked grouping in lipids is similar to the enzymatic mechanism described for the hydroxylation of phenylalanine. Fatty aldehydes produced via the cleavage reaction can be either oxidized to the corresponding acid or reduced to the alcohol by appropriate enzymes.

Alkyl cleavage enzyme activities are highest in liver and intestinal tissue, whereas most other cells/tissues possess very low activities. Tumors and other tissues that contain significant quantities of alkyl lipids generally have very low alkyl cleavage enzyme activities, which is consistent with the overall premise that the level of ether-linked glycerolipids is inversely proportional to the activity of the alkyl cleavage enzyme.

Structural features of glycerolipid substrates utilized by the alkyl cleavage enzyme are (a) an O-alkyl moiety at the sn-1 position, (b) a free hydroxyl group at the sn-2 position, and (c) a free hydroxyl or phosphobase group at the sn-3 position. If the

hydroxyl group at the *sn*-2 position is replaced by a ketone or acyl grouping, or when a free phosphate is at the *sn*-3 position, the *O*-alkyl moiety at the *sn*-1 position is not cleaved by the Pte·H₄-dependent monooxygenase. Thus, 1-alkyl-2-lysophospholipids (e.g., lyso-PAF) are substrates for the cleavage enzyme, but they are attacked at much slower rates than are alkylglycerols.

7.2.2. Plasmalogenases

Plasmalogenases (Fig. 13B) are capable of hydrolyzing the O-alk-1-enyl grouping of plasmalogens or lysoplasmalogens. The products of this reaction are a fatty aldehyde and either 1-lyso-2-acyl-sn-glycero-3-phosphoethanolamine (or choline) or sn-glycero-3-phosphoethanolamine (or choline), depending on the chemical structure of the parent substrate. Plasmalogenase activities have been described in microsomal preparations from liver and brain of rats, cattle, and dogs, but their biological significance is poorly understood.

7.2.3. Phospholipases and lipases

In general, the *sn*-2 and *sn*-3 ester groupings associated with either the alkyl or alk-1enyl glycerolipids are hydrolyzed by lipolytic enzymes with the same degree of substrate specificity as their acyl counterparts (Fig. 14). However, the ether linkages themselves are not hydrolyzed by lipases or phospholipases and the presence of an ether linkage at the *sn*-1 position of the glycerol moiety generally reduces the overall reaction rate to the extent that certain lipases have been successfully used to remove diacyl contaminants in the purification of some ether-linked phospholipids. The only lipolytic enzyme (other than those that cleave the ether linkages) known to exhibit an absolute specificity for ether-linked lipids is lysophospholipase D. The uniqueness of lysophospholipase D is that it exclusively recognizes only 1-alkyl-2-lyso-*sn*-glycero-3-phosphobases or 1-alk-1-enyl-2-lyso-*sn*-glycero-3-phosphobases as substrates; thus, lyso-PAF is a substrate for this novel enzyme (Fig. 14, Reaction II).

7.3. PAF and related bioactive species

7.3.1. Acetylhydrolase

PAF acetylhydrolase (AH) enzymes (Fig. 14, Reaction I) are a specific group of Ca^{2+} independent phospholipases A₂ that remove the acetyl moiety at the *sn*-2 position of PAF [29–31]. Mammalian PAF-AHs can be classified into intracellular and extracellular types. Intracellular PAF-AHs consist of at least three groups of enzymes, namely, PAF-AH I, PAF-AH II, and erythrocyte-type PAF-AH. Extracellular PAF-AH occurs as plasma AH.

The erythrocyte enzyme appears to be a homodimer comprised of the 25-kDa polypeptide and is different from PAF-AH II. PAF-AH is a serine esterase and requires reducing agents for maximal activity. The most likely role of the erythrocyte PAF-AH in vivo is to hydrolyze the products of oxidative fragmentation of membrane phospholipids.

PAF-AH I, rich in brain and exclusively located in cytosols, is an unusual G-proteinlike $(\alpha_1/\alpha_2)\beta$ heterotrimer complex composed of 45(β)-, 30(α_2)-, and 29(α_1)-kDa



Fig. 14. Catabolism of PAF and its metabolites can be catalyzed by the following enzymes: (I) PAF acetylhydrolase, (II) lysophospholipase D, (III) phosphohydrolase, (IV) phospholipase C, (V) CoA-*independent* or CoA-*dependent* transacylase, and/or (VI) alkylacetylglycerol acetylhydrolase. The O-alkyl linkage in those products that contain free hydroxyl groups can be cleaved by (VII) the O-alkyl Pte·H₄-dependent monooxygenase.

subunits. The tertiary fold of α_1 subunit is similar to that found in p21^{ras} and other GTPases. The active site is made up of a trypsin-like triad of Ser 47, His 195, and Asp 193. A sequence of ~30 amino acids adjacent to the active serine residue exhibits significant similarity to the first transmembrane region of the PAF receptor. The catalytic 30(α_2)-kDa subunit is highly homologous (63.2% identity) to that of the 29(α_1)-kDa subunit, especially (86%) in the catalytic and PAF receptor homologous domains.

The 45(β) kDa subunit, which is not essential for catalytic activity, exhibits striking homology (99%) with a protein encoded by the causal gene (*LIS-1*) for the onset of Miller–Dieker lissencephaly, a human brain malformation manifested by a smooth cerebral surface and impaired neural migration. In addition, the 45-kDa subunit contains a 7-tandem WD-40 repeat in its primary structure. This repeat is thought to be important for interactions with other protein components, especially with pleckstrin-homology (PH) domains. Therefore, the hydrolysis of PAF may induce conformational changes in the heterotrimeric PAF-AH I complex that affect the ability of the 45-kDa subunit to interact with cytoskeletal proteins. Furthermore, the α_1 subunit appears to be expressed specifically in neurons of fetal and neonatal brain of rats. Significant levels of the α_1 subunit are not expressed in any adult rat tissues. In contrast, α_2 and β transcripts and proteins are almost constantly expressed from fetal stages through adulthood. The catalytic subunits switch from the α_1/α_2 heterodimer to the α_2/α_2 homodimer and along with the above-mentioned data suggest that PAF-AH I in brain is involved in brain development through regulation of neuronal migration.

PAF-AH type II (PAF-AH II), expressed most abundantly in liver and kidney, is a monomeric 40-kDa protein and a member of the serine esterase family. This enzyme exhibits broader substrate specificity than PAF-AH I. PAF-AH II hydrolyzes oxidized phospholipids as effectively as PAF, whereas PAF-AH I is more specific for PAF. Furthermore, unlike PAF-AH I, PAF-AH II is distributed in both the membrane and soluble fractions.

PAF-AH II is a *N*-myristoylated enzyme, the first reported among lipases and phospholipases. It translocates between cytosol and membranes in response to the redox state. When overexpressed in cells, it protects against oxidative stress-induced cell death. PAF-AH II may function as an antioxidant phospholipase and promote the hydrolysis of oxidized phospholipids produced during reactive oxygen species-induced apoptosis.

Plasma PAF-AH is unique since it is mainly associated with the high density and low density lipoproteins. Plasma PAF-AH also hydrolyzes PAF and structurally related oxidized phospholipids with up to 9 carbon *sn*-2 acyl chains. The cDNA for this enzyme encodes a 44-kDa secretory protein that contains a typical signal sequence and a serine esterase/neutral lipase consensus motif GXSXG. Serine 273 (of the GXSXG motif), Asp 296, and His 351 are required for catalysis. Plasma PAF-AH displays \sim 40% homology with intracellular PAF-AH II, but little homology/similarity with PAF-AH I over the whole amino acid sequence.

Pretreatment of animals with recombinant plasma PAF-AH blocks PAF-induced inflammation. Furthermore, deficiency of plasma PAF-AH is an autosomal recessive syndrome that is associated with severe asthma in Japanese children. A point mutation of exon 9 of the plasma PAF-AH gene results in the production of an inactive protein. In addition, an increase in enzyme activity has been reported in humans or rats with hypertension, and in the plasma of patients with atherosclerosis. The level of plasma PAF-AH decreased markedly near the end of pregnancy in rabbits. It has been proposed that this is a component of the mechanism for initiating the onset of labor. Since PAF stimulates the contraction of uterine muscle, a decrease in PAF-AH activity will allow the accumulation of PAF

8. Metabolic regulation

Regulatory mechanisms that control the metabolism of ether-linked lipids are still poorly understood. In fact, most progress in this area has concerned PAF metabolism, primarily because of the high degree of interest in this potent mediator. Nevertheless, a variety of factors are known to influence the overall rates of ether lipid metabolism, but such studies have mainly been of the descriptive type and none have addressed the molecular enzymatic mechanisms involved. Regulatory controls that must be considered in the metabolism of ether-linked lipids are those that influence (a) the enzymes responsible for catalyzing the biosynthesis and catabolism of the ether lipid precursors (fatty alcohols and dihydroxyacetone-P), (b) alkyldihydroxyacetone-P synthase which is responsible for the synthesis of alkyldihydroxyacetone-P, and (c) branch point enzymes, e.g., those steps that utilize diradylglycerols.

Glycolysis plays an important role in controlling the levels of ether lipids at the precursor level. For example, the high glycolytic rate of tumors generates significant quantities of dihydroxyacetone-P, which could explain the relatively high levels of ether lipids found in cancerous cells.

Factors responsible for the regulation of biosynthetic and catabolic enzyme activities that catalyze specific reaction steps in the metabolic pathways for ether-linked lipids appear to be very complex. Although the rate-limiting steps are poorly understood, two important intermediary branch points in the biosynthesis of ether-linked lipids involve 1-alkyl-2-lyso-sn-glycero-3-P and 1-alkyl-2-acyl-sn-glycerols. The 1-alkyl-2lyso-sn-glycero-3-P can be ultimately converted to either PAF via de novo route or to 1-alkyl-2-acyl-sn-glycerols following an acylation and dephosphorylation step. The alkylacylglycerols represent a branch point since they are the direct precursors of plasmanylcholines, plasmanylethanolamines, and alkyldiacylglycerols. Conditions that influence either branch point would have profound effects on the proportion of the different types of ether-linked lipids formed. Since the choline- and ethanolaminephosphotransferases appear to be able to utilize both diacyl- and alkylacyl-glycerols, it is apparent that the availability of specific diradylglycerols is crucial in controlling the diacyl and alkylacyl species composition of membranes. Most of the catabolic enzymes of ether lipid metabolism have received far less attention than those associated with the biosynthetic pathways.

Studies of the regulation of PAF metabolism are still in the early stages of development. Rate-limiting steps in the de novo pathway of PAF biosynthesis are the acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-P acetyltransferase and the cytidylyltransferase that forms CDP-choline for the cholinephosphotransferase catalyzed step (fig. 10, Chapter 8). Any factor that stimulates these rate-limiting reactions (e.g., activation of cytidylyltransferase by fatty acids) is also known to enhance the de novo synthesis of PAF.

In the remodeling pathway, it is clear that arachidonic acid can influence the formation of PAF at the substrate level since cells depleted of alkylarachidonoylglycerophosphocholines lose their ability to synthesize PAF. Therefore, the transacylase/phospholipase A_2 step (Fig. 9, Reaction II) as well as a specific phospholipase A_2 (Fig. 9, Reactions I or III) can be rate-limiting. Regulation of the acetyl-CoA:lyso-PAF acetyltransferase in the remodeling pathway (Fig. 9, Reaction IV) appears to be controlled by a phosphorylation/phosphohydrolase system.

Acetylhydrolase and other catabolic enzymes in PAF metabolism also have an important regulatory role in controlling PAF levels since it is known that the activity of acetylhydrolase can drastically change during various diseases, pregnancy, and macrophage differentiation.

9. Functions

9.1. Membrane components

Cellular functions of ether-linked glycerolipids are especially poorly understood, but their ability to serve as both membrane components and as cellular mediators is now well established. Both the alkyl and alk-1-enyl phospholipids that contain long-chain acyl groups at the *sn*-2 position appear to be essential structural components of many membrane systems. Some species of the ether lipids associated with membranes act as storage reservoirs for polyunsaturated fatty acids. The apparent protective nature of ether-linked groups against lipolytic actions is reflected by their ability to slow the rate of hydrolysis of acyl moieties at the *sn*-2 position by phospholipase A₂. The preferential sequestering of polyunsaturated fatty acids in ether-linked phospholipids has been observed even in essential fatty acid deficiency. The plasmalogens of inflammatory cells are highly enriched in arachidonate; in human neutrophils 80% of the cellular arachidonate is found in the plasmenylethanolamine fraction. There is one report with photosensitized cells exposed to long wavelength ultraviolet radiation that has suggested that plasmalogens might have a role in protecting membranes against certain forms of oxidative stress. However, the true function(s) of plasmalogens remains a puzzle.

9.2. Cell mediators

The multifaceted responses generated by PAF in vivo and in target cells and the ubiquitous distribution of PAF-related enzymes in mammalian cells has emphasized the important role of bioactive ether-linked lipids as diverse regulators of metabolic and cellular processes. Also, identification of the PAF receptor as a member of the general family of G-protein-coupled receptors further strengthens the potential importance of PAF as a mediator in cell signalling pathways. Activation of phosphatidylinositol-specific phospholipase C by the binding of PAF to its receptor elevates the intracellular levels of Ca²⁺ and diacylglycerols, events that activate protein kinase C (Chapter 12). The latter catalyzes the phosphorylation of specific proteins and it is clearly documented that 20- and 40-kDa proteins are phosphorylated in PAF-treated rabbit platelets. However, an array of kinase cascades and cellular alterations need to be considered in any proposed biochemical mechanism for explaining the diverse actions of PAF.

10. Future directions

There are many unanswered questions about the dual role that ether lipids serve as membrane components and as cellular signaling molecules. Although it is clear that arachidonic acid is closely associated and tenaciously retained by ether lipids in membranes, even in essential fatty acid deficiency, much remains to be elucidated about the enzymatic systems and regulatory controls that affect the release of this sequestered pool of arachidonic acid for its subsequent conversion to bioactive eicosanoid metabolites. The mechanisms that account for the synergistic actions of PAF and eicosanoids are still poorly understood. In addition, the significance of ether lipids as a dietary nutrient has received little attention even though they occur in a variety of foods and it is known that ether lipid supplements are readily incorporated into cellular lipids.

It is noteworthy that organisms living in harsh environments of high temperatures or high salt and low pH contain only ether lipids suggesting that they serve as the Teflon of lipids. Invasive cells such as neutrophils and eosinophils also have high levels of ether lipids. Based on these observations it is tempting to speculate that the high levels of ether lipids found in most tumor cells may contribute to their invasiveness.

Now that the alkyl synthase has been cloned and sequenced and shown to contain a flavin, further elucidation of the mechanism of ether bond synthesis is anticipated. Despite the advances made in cloning and sequencing of the PAF receptor, the exact mechanisms for explaining how PAF participates in signal transduction, the generation of second messengers, and gene expression remain poorly understood. Moreover, even the binding site of PAF to its receptor has not yet been rigorously identified and the presence of intracellular PAF binding sites still are unresolved issues. Certainly, the significance of PAF in physiological and disease processes needs to be more firmly established.

A major enigma is the function of plasmalogens. Despite the large quantities of ethanolamine plasmalogens found in nervous tissue and other cells, their cellular role or the molecular mechanism and regulatory controls for the alkyl desaturase responsible for their formation are still unknown. The alkyl desaturase has yet to be purified, cloned and carefully compared to fatty acid desaturases. Likewise, the biosynthesis of choline plasmalogens is still not fully understood, although compelling evidence exists to indicate that they originate from ethanolamine plasmalogens via remodeling mechanisms.

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