The Biosynthesis and Functions of Plasmalogens

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Summary  The biosynthesis of plasmalogen (alkenyl acyl glycerophosphoryl choline or ethanolamine) is independent of diacyl glycerophospholipid synthesis and requires the coordinated activities of peroxisomal, cytosolic, and endoplasmic reticulum enzymes. This would indicate that the plasmalogens may have a unique role(s) to play in cellular functions, one different from that of the diacyl glycerophospholipids. Several functions have been assigned to plasmalogens such as antioxidants, arachidonic acid storage terminals, receptor mediators, substrate for the biosynthesis of platelet-activating-factor (PAF), and determinant of physicochemical properties of biomembranes. Except for serving as a substrate for biologically active PAF biosynthesis, none of the proposed functions have been fully validated. The purpose of this review article is to provide up-to-date knowledge of the biosynthetic pathway and functions of plasmalogens.

Key Words: plasmalogens, alkyl-acylphosphoglycerides, alkenyl-acylphosphoglycerides, functions, ether-linked phospholipids

Many years have passed since ether-linked phospholipids were first discovered in tissue membrane. Their actual structure, however, was not identified until the 1960's [1]. Two sub-classes exist, differing in the moiety present at the sn-1-position of the glycerol backbone [2]. A saturated O-alkyl linkage and an alpha, beta-unsaturated O-alkenyl linkage (vinyl ether linkage) are the distinguishing features of these two molecules. This review will focus on the latter product, alkenyl acyl glycerophosphoryl ethanolamine (choline), which has also been given the trivial name of plasmalogen. As mentioned, both ethanolamine and choline may be present in the plasmalogen molecule at the sn-3 position. Ethanolamine

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plasmalogens are more abundant in most cells, but choline plasmalogens appear to have a higher turnover rate in the cell [3], indicating that both may have important functional role [4].

Plasmalogens are structural components of lipid bilayers [5]. They are found in varying degrees in most mammalian tissue. Some cells that are especially rich in plasmalogens (≥30% of the total ethanolamine phospholipids) are found in compartments such as the heart, brain, and skeletal muscle [6]. Neutrophils [7] and macrophages [4] also have been shown to contain high amounts of plasmalogens in their membranes.

Originally plasmalogens were just thought to be metabolized glycerolipids, but it was later discovered that ether lipids have their own unique pathway for biosynthesis. It would only be natural, therefore, to assume that plasmalogens also have a distinct functional role in the cell. It has been hypothesized that plasmalogens may act as antioxidants, polyunsaturated fatty acid storage terminals, receptor mediators and stabilizing structures in the cell. However, more research is imperative in this area if the question of plasmalogen function is to be answered satisfactorily.

BIOSYNTHESIS

The biosynthetic pathway leading to the synthesis of plasmalogens is not as simple as that of diacylphosphoglycerides but involves complex co-ordination of peroxisomes, cytosol, and endoplasmic reticulum. The laboratories of Hajra, Freidberg, and Snyder discovered these pathways in the late sixties and early seventies (Fig. 1) [for reviews see 8–11]. The following three areas will be clarified in this section: a) peroxisome function; b) the CDP-ethanolamine step, and c) the final step in plasmalogen biosynthesis.

The first two enzymatic steps in plasmalogen biosynthesis occur in the peroxisome of the cell [12–14]. Peroxisomes introduce the vinyl ether bond, i.e., esterify a fatty alcohol with dihydroxyacetone phosphate or glycerol-3-phosphate. The role of peroxisomes is very evident in children with cerebro-hepato-renal (Zellweger) syndrome. Zellweger patients lack peroxisomes and therefore have very depressed levels of plasmalogens in their cells [15]. Schrakamp et al. [16] demonstrated the importance of peroxisomes in plasmalogen synthesis by growing Zellweger fibroblast cells in a medium containing [14C]hexadecanol, a long-chain alcohol involved in the second step of plasmalogen synthesis. There was decreased de novo synthesis of plasmalogens but this could be corrected when [3H]octadecylglycerol was added to the medium. With the vinyl ether bond already in place, octadecylglycerol could take the 3'-7 (Fig. 1) pathway to synthesize plasmalogens. This by-passes the peroxisome. Studies carried out by Gitsham et al. [17] support this view. Mucosal cells from small intestine of guinea pigs were examined to determine where the first two enzymes in the biosynthesis of plasmalogens were situated. Organelles were well separated by a metrizamide gradient and enzyme

activity was examined. It was found that the first two enzymes needed in the synthesis of plasmalogens, alkyldihydroxyacetonephosphate and dihydroxyacetonephosphate acyltransferase, had peak activities corresponding to the activity of catalase. As catalase is the marker enzyme for peroxisomes, or in the intestinal mucosa, microperoxisomes, it was shown that the first two steps of plasmalogen biosynthesis do occur in the peroxisome. Previously there had been some confusion as to the actual location of the number I and number II enzymes. Poor separation of the organelles was presumably the problem [11]. Since peroxisomes and lysosomes would sediment together in the same fraction, it was not known exactly in which organelle the plasmalogen enzyme activity was occurring. After biosynthesis in the peroxisome is complete, 1-alkyl DHAP is converted to 1-alkyl-2-acyl-sn-glycerol-3-P. At this point the enzymes needed to continue this biosynthetic pathway are situated on the endoplasmic reticulum. This is where most lipids are synthesized and transported to different intracellular membranes [1].

Research carried out on the CDP-ethanolamine pathway dates back to the late 1950’s when Kiyasu and Kennedy [18] demonstrated that 1-alk-1’-enyl-2-sn-glycerol was converted to 1-alk-1’-enyl-2-acyl-GPE by the use of this pathway. The
enzyme involved in this conversion is the same one used to generate the diacylglycerol analogs of plasmalogens. Since ethanolamine glycerophosphate (EGP) synthesis can occur via: (a) the CDP-ethanolamine pathway, (b) the decarboxylation of serine glycerophospholipids (SGP), and (c) a Ca\(^{2+}\)-dependent base exchange of ethanolamine with pre-existing phospholipids [19], it was postulated that plasmalogens could be synthesized in these various ways as well. Arthur and Page [19] showed that plasmalogens in rat heart, kidney, and liver were synthesized by the CDP-ethanolamine pathway. However, no significant incorporation of \(^{3}\)H-serine into ethanolamine plasmalogens was observed, indicating that serine glycerophosphate (SGP) did not contribute significantly to the synthesis of ethanolamine plasmalogens. This means that decarboxylation or base-exchange reaction, which would release ethanolamine for plasmalogen synthesis, does not occur to any appreciable degree in some cells. This conflicts with an earlier study [20] that demonstrated Ca\(^{2+}\)-base-exchange reactions had occurred in rat brain microsomes to form ethanolamine and serine plasmalogens. It must be emphasized that various tissues may utilize different pathways, at this point, to synthesize plasmalogens. The reason for this, however, is not fully understood [19].

As mentioned before, the CDP-ethanolamine pathway synthesizes both plasmalogens and EGP. Different tissues seem to preferentially utilize the CDP-ethanolamine pathway to synthesize one subclass over the other. For example, in rats, the kidney appears to preferentially synthesize ethanolamine plasmalogen, whereas in the liver EGP is the main phospholipid made [19]. The regulation of which subclass is synthesized is also not known but it may be determined by ethanolamine phosphotransferase, the enzyme that catalyzes the committed step in EGP synthesis [19].

The final enzymatic step in the biosynthesis of plasmalogens was established in two different laboratories [21–23]. Cell-free systems were set up to synthesize plasmalogens [24, 25]. By the use of different marker compounds, the final step in the biosynthesis of plasmalogens was discovered. 1-Alkyl-2-acyl-GPE is converted to 1-alk-1'-enyl-2-acyl-GPE by a desaturation step introducing a double bond between carbons 1 and 2 of the alkyl moiety. 1-Alkyl-2-acyl-GPE is the only known precursor. If 1-alkyl-2-lyso-GPE is introduced to the system it is rapidly acylated before it is desaturated [26]. As well, 1-alkyl-2-acyl-GPE can not be desaturated by this system. 1-alkyl-2-acyl-sn-glycero-3-phosphoethanolamine desaturase is the enzyme responsible for this conversion. It is a microsomal, mixed-function oxidase, that requires molecular oxygen, NADH, and cytochrome b\(_5\) to function. This enzyme is very similar to the stearoyl-CoA desaturase enzyme [27, 28], i.e., it is inhibited by KCN but not by CO, yet it is distinct. This was demonstrated by Lee et al. [29]. They noted these two enzymes had different levels of activity in response to a fat-free diet. Only the activity of the stearoyl-CoA desaturase enzyme increased on the fat-free diet, while there was no increase noted in the desaturation of 1-alkyl-2-acyl-GPE.

The synthesis of ethanolamine plasmalogens by this route is well accepted.
The question still remains as to how choline plasmalogens are synthesized. They can not be desaturated by the same enzyme that makes ethanolamine plasmalogens, since this desaturase enzyme is specific for 1-alkyl-2-acyl-GPE. Synthesis of choline plasmalogens has been proposed to occur through phospholipid-N-methylation of ethanolamine plasmalogens [30]. Mozzi et al. [31] observed the methylation of ethanolamine plasmalogens as well as acid-stable GPE to the corresponding choline phospholipids in chick neuron cells labelled with radioactive ethanolamine and methionine. Hack and Helmy [32], however, observed no amount of monomethyl- or dimethyl-PE plasmalogen in the heart tissue they analyzed. They concluded that there must be a desaturase that is specific for 1-alkyl-2-acyl-GPC. This would mean that the main route for the synthesis of choline plasmalogens would be the same as for the synthesis of platelet-activating factor (PAF); but instead of 1-alkyl-acyl-GPC being acetylated to PAF, it would be desaturated to choline plasmalogen. One of the problems in determining the fate of choline plasmalogens is that they are found in such small amount in the cell due to their high turnover rate [31]. It is true that most of the synthesis of choline phospholipids occurs via the CDP-choline pathway (90%) rather than by progressive methylation (2.5%) [33], but this low rate of methylation may be enough to replenish the choline plasmalogen pool via ethanolamine plasmalogens. Finally, it is also likely that the activities of alkyl-acyl-GPE or alkyl-acyl-GPC and N-methylation of alkenyl-acyl-GPC to synthesize alkenyl-acyl-GPC is tissue specific.

**FUNCTIONAL ROLE**

*Cell structure*

There has been much speculation in recent years as to the functional role of plasmalogens; however, the reason for their predominance in tissue membranes still remains a mystery [34]. Plasmalogens do have some unique physical properties that may affect membrane function [35]. Just by introducing the alk-enyl ether bond, versus the acyl ester bond in position 1 of the diacyl analog, one radically lowers the lamellar to the hexagonal phase transition (by 38°C) while not vastly altering the \( T_m \) (liquid-crystalline phase transition), which is only 4 degrees lower for the plasmalogen. Ethanolamine plasmalogens are even able to stay in the non-bilayer (non-lamellar) phase in the presence of high amounts of lipids (38 mol%) that prefer the bilayer arrangement [36]. This may be important for the temporary, local formation of non-bilayer phases which can occur during membrane fusion or during interactions of the membrane with external factors such as ions of proteins [36]. Plasmalogens contribute to the organization of the membrane, but until the differences in molecular geometry can be fully elucidated, it will be difficult to know exactly how its atypical structure affects the biological workings of the cell [34].
**Arachidonate regulation**

Ethanolamine plasmalogens have also been indicated a storage terminals for polyunsaturated fatty acids (PUFA), especially arachidonic acid [37, 38]. This may have important implications, because arachidonic acid is known for its role in the biosynthesis of eicosanoids [39]. Eicosanoids regulate vascular smooth muscle cell contractile state, hypertrophy, and proliferation [38]. Therefore, plasmalogens may have an important function in condition such as atherosclerotic plaques, smooth muscle hypertrophy, smooth muscle cell injury [38], and inflammatory disease. Ford and Gross [38] completed a study on the release of arachidonic acid from rabbit vascular smooth muscle after stimulation with the agonist angiotensin II. They found that more than 60% of the arachidonic acid released from ethanolamine glycerophospholipids came from the ethanolamine plasmalogen moiety. A membrane-associated calcium-independent phospholipase A₂ is the enzyme that cleaves the sn-2 fatty acid (i.e., arachidonic acid) from the plasmalogen molecule [40]. This membrane-associated phospholipase A₂ has different kinetic characteristics than the cytosolic, calcium-dependent phospholipase A₂. The membrane-associated phospholipase is much more specific for plasmalogen catabolism [41]. The activity of this phospholipase is rapidly reversible, indicating that it may be a significant biological regulator [41]. Ford et al. [41] suggested that the role that these phospholipases have as enzymic mediators or initiators of cellular response to physiologic perturbations in mammalian cells merits consideration. For this reason Ford and Gross [38] advise the use of caution when interpreting data on arachidonate release, because ethanolamine glycerophospholipids may be using arachidonic acid for signal transduction rather than for eicosanoid production.

When plasmalogens are catabolized by phospholipase A₂ the resulting product is the 1-O-alk-1'-enyl-2-lyso-sn-glycero-3-phosphoethanolamine. Tessner and Wykle [42] have shown that [³H]acetate can become incorporated into this molecule in stimulated neutrophils. An ethanolamine plasmalogen analog of platelet-activating factor (PAF) is the resulting product. PAF is the most potent lipid mediator yet discovered, but it is not known if the ethanolamine plasmalogen analog has any cellular function or significance [43]. It may act synergistically with PAF, but preliminary data has not been conclusive [43].

Lysoplasmalogens have also been indicated as acceptors for arachidonic acid in the remodeling pathway that leads to the biosynthesis of PAF [44, 45]. The remodeling pathway involves cleavage of the acyl group from the sn-2 position of 1-alkyl-2-acyl-sn-glycero-3-phosphocholine by phospholipase A₂. An acetyl group is then added at the sn-2 position, resulting in the formation of PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine). Uemura et al. [44] postulated that the catalytic activities responsible for the remodeling pathway (phospholipase A₂/transacylase) could reside in a single protein or as a tightly integrated complex known as CoA-independent transacylase [44]. Lysoplasmalogens involved in this pathway could be important in the reincorporation and subsequent redistribution of...
arachidonic acid during stimulation, thus affecting two different types of biologically active mediators (PAF and eicosanoid metabolites) \[43, 45\], making it very economical for the cell.

Addition of 1-alkenyl(alkyl)-GPE to human leukocyte cell cultures also induces the production of PAF \[46\]. Sugiura et al. \[46\] speculated that ethanolamine plasmalogen may increase the availability of lyso-PAF for PAF formation within the cell by causing the degradation of cellular alkylarachidonoyl-GPC by the CoA-independent transacylase pathway. However, there is the possibility that 1-alkenyl(alkyl)-GPE is first metabolized to lyso-PAF and then converted to PAF \[46\]. Plasmalogens may be acting as an inactive storage precursor for the synthesis of PAF.

Fish oils (n-3) have been of interest recently due to their apparent ability to decrease the occurrence of coronary heart disease. Studies have been done to see if the composition of the PUFA at the sn-2 position of the phospholipids can change in volunteers on a diet supplemented with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) \[47\]. And in a study by Blank et al. \[4\] macrophage-like cells were grown in medium supplemented with EPA and DHA. It was found that the largest change in acyl group composition occurred in the ethanolamine plasmalogen fraction. Both EPA and DHA replaced oleate at the sn-2 position of the glycerol backbone. DHA was further incorporated into the sn-2 position (up to 46%), displacing one-third of the arachidonate. This effect was also observed in human subjects studied by Holub and Skeaff \[47\], who examined the effect that consuming fish oil supplements would have on the composition of PUFA in plasmalogens of human platelets. They found that ethanolamine plasmalogens contained the majority of EPA and docosapentaenoic acid (DPA) compared with the diacyl analog. Arachidonic acid-phospholipids and platelet reactivity were reduced in these people, as well. If humans are on a diet supplemented with fish oil, DHA, DPA and/or EPA may be displacing arachidonic acid at the sn-2 position of plasmalogens, and, therefore, less arachidonic acid will be released. Since n-3 fish oils are metabolized in a similar way as arachidonate \[48\], it has been proposed that they reduce some of the detrimental effects of arachidonate metabolites in certain diseases by competing with arachidonate for enzymes involved in eicosanoid formation \[39\]. For example, fish oil metabolites (i.e., TxA\(_3\), PGA\(_3\), and LTB\(_3\)) are less effective than arachidonate metabolites (i.e., TxA\(_2\), PGA\(_2\), and LTB\(_4\)) in having a platelet-aggregating effect in the body. This would explain the depressed platelet reactivity found in subjects consuming large amounts of fish oils \[37, 47\].

**Myocardial ischemia**

Further studies along these lines have proposed a link between lethal ischemic damage to myocardial tissue and plasmalogen degradation \[49\]. Ischemic damage is accompanied by a build-up of amphiphilic lysophospholipids. This is a main determinant of electrophysiologic dysfunction in the ischemic myocardium. Lyso-
phospholipid production has been attributed to the action of phospholipase \( A_1 \) and/or \( A_2 \), which cleave the 1-alk-enyl and/or the 2-acyl group on the plasmalogen. In both cases lysophospholipids are produced [50]. There is another way in which amphiphilic products may accumulate, and that is by free radical oxidation of the plasmalogen [50]. Plasmalogens have a vinyl-ether linkage that can be easily oxidized by \( 1^2O_2 \) [49]. Morand et al. [50] demonstrated this phenomenon in Chinese hamster ovary cells. By observing the products formed during UV irradiation (2-monoacyl-glycerophosphoethanolamine, formic acid, and pentadecanal), they proposed that cyclo-addition of singlet oxygen or a \( 1^2 \)-allylic hydroperoxide derivative of the vinyl ether moiety brought on by singlet oxygen or radical-initiated oxidation was responsible for the oxidation of the vinyl ether linkage in plasmalogens. In biological systems \( 1^2O_2 \) can be generated by invading white blood cells [51] or by metal-catalyzed oxidation of superoxide [52]. There is also evidence that during times of vascular inefficiency, as in ischemia, oxidative bursts occur [53]. This would aid in the production of free-radicals, as well. The reason that plasmalogens may be cleaved is that they are acting as an antioxidant in the plasma membrane [50, 54], to protect the functioning of other cell components that are more difficult to replace, such as histidyl, methionyl, and tryptophanyl residues of membrane-spanning proteins [50, 54]. The significance of plasmalogens acting as antioxidants does not stop here. Such action may be implicated in the area of aging, as well.

Antioxidant

Some theories link aging to cellular structure and function [55]. It is thought that loss of the structural integrity of the cell leads to cellular deterioration and ultimately to whole-body deterioration. One way in which cells may lose their viability is through free-radical damage. If plasmalogens are important antioxidants in the cell, with age their amount could be decreased, allowing an increase in free-radical damage to the cell. Brosche et al. [56] set out to answer this question. They found that there was a significant difference in the amount of plasmalogens present in red blood cells and plasma phospholipids between people \( \geq 70 \) years old and younger people. As donor age increased, plasmalogen levels declined. Rouser and Yamamoto [57] looked at ethanolamine plasmalogen content in the brain and also found an age-contingent factor. There was a steep increase in plasmalogen content in the brain just after birth. This has been linked with the rapid production of myelin in the first few weeks of life [32]. Increased plasmalogen content in the brain was constant until the age of thirty, at which point the levels started to decrease. By the age of eighty, the quantity of plasmalogens in the brain had decreased to levels below that of a one year old. What the link is between aging and plasmalogen content in cells is still not known.

Atherosclerotic aortas also increase with age, as the plasmalogen content of the aortic cells decreases. Buddecke and Andresen [58] did note a drop in the level of plasmalogen found in the blood of donors with atherosclerotic plaques when
this level was compared with that of a "normal" population of the same age. A recent study done by Vance [59] makes a connection between the ability of plasmalogens to be antioxidants in cell membranes and to act as protective agents in atherosclerosis. Atherosclerotic lesions are thought to originate from macrophages [60]. Macrophages have a scavenger receptor that recognizes chemically altered LDL molecules. Such modifications can be accomplished by singlet oxygen molecules. The macrophages accumulate LDL, and consequently cholesterol esters, leading to the development of atherosclerotic plaques [59]. Vance postulated that the function of plasmalogens in lipoproteins may be to prevent the oxidation of the cell membrane and thereby prevent the accumulation of LDL and cholesterol in macrophages that have atherogenic potential. This study looked at the ability of rat hepatocytes to secrete ethanolamine plasmalogen as a component of newly synthesized lipoproteins [59]. Plasmalogens were indeed found in all lipoprotein fractions at levels of 30% of the ethanolamine-containing phospholipids. The premise would be that, as people age and have a decreased ability to synthesize plasmalogens, they may also have an increased risk of developing atherosclerosis. It is interesting to note, at this point, that hypolipidemic drugs affect total plasmalogen content in the intestine of guinea pigs. A threefold increase was noted after short-term administration of the drug [17]. This rise in plasmalogen content may increase the amount incorporated into lipoproteins and in turn decrease the abnormal accumulation of cholesterol by macrophage cells.

CONCLUSIONS AND FUTURE DIRECTIONS

If plasmalogens are important for the body to function properly, the question then becomes: how is the biosynthesis of plasmalogens regulated? Can one increase the production of plasmalogens? Although the main biosynthetic pathway used to assemble plasmalogens has been well established, there may be other less known pathways that are very important in the production of plasmalogens. The discovery of novel pathways of biosynthesis and catabolism will assist researchers in elucidating the functional role that plasmalogens possess in the cell, as well. The importance of certain body tissues being rich in ether-linked phospholipids needs to be explored further. Whether the levels and biosynthesis of plasmalogens are altered in the diseased state and if these are involved in the pathophysiology of certain diseases are questions that need to be answered. Structural and biochemical roles have been hypothesized but with no degree of certainty, indicating the need for more research in this area. If, as suggested, plasmalogens have anti-oxidant properties, then their role in the prevention of oxidation of LDL, atherosclerosis, and cancer deserves further attention. In any discussion on phospholipids, plasmalogens should be given special consideration.

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