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Review

Plasmalogens: biosynthesis and functions

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Contents

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1. Introduction

Possibly of all the common phospholipids, least is known about plasmalogens. Although plasmalogens represent one in every five phospholipids in the human body, one would be hard-pressed to find an adequate description of their structure or biosynthesis in Biochemistry or Cell Biology textbooks. This lack of knowledge is, in part, due to the fact that plasmalogens are a subclass of phospholipid and, unlike other phospholipids, are not defined by the polar head group. It is not possible to separate them from other phospholipids using the conventional separation systems; they must be chemically modified first or rather sophisticated technologies must be used, adding significant time and effort to analyses. Another complicating factor is the fact that they are very sensitive to acid; the addition of acid to lipid extraction, often used to ensure protonation and complete extraction of acidic lipids, also ensures degradation of plasmalogens. Given these problems, one has to have a vested interested in their study.

Fifteen years ago few scientists, even in the lipid field, were familiar with plasmalogens. However, their abundance in tissues such as brain and heart, and their links with certain pathologies and with human genetic disorders has stimulated interest in these molecules. Our intention is to give an overview of the plasmalogen field with emphasis on more recent data concerning plasmalogen biosynthesis and function in animals.

2. Structures

Animal cell glycerophospholipids are most commonly known to consist of a glycerol backbone, which contain long-chain fatty acids attached at the *sn*-1 and *sn*-2 through ester linkages. The *sn*-3 position is adorned by one of a variety of head groups attached through a phosphodiester linkage (Fig. 1). However, within each of the headgroup classes can be found species that bear an ether-linked alkyl chain at the *sn*-1 position instead of the ester-linked fatty acid. Some of the ether-linked phospholipids also display a *cis* double bond on the alkyl chain, adjacent to the ether bond, forming a "vinyl-ether linkage". It is this latter species that is termed a plasmalogen. Any glycerophospholipid displaying the vinyl ether at the *sn*-1 position is referred to as plasmalogen. It is this vinyl ether double bond that gives plasmalogens their identity and their unique sensitivity to acid, mercury cation, and reactive oxygen species.

Plasmalogens were first identified by Feulgen and Voit in 1924 [1] as fuschin-sulfurous acid staining compounds generated in tissue sections treated with acid or mercuric chloride as a part of a routine nuclear staining technique. Since it was known that aldehydes reacted with fuschin-sulfurous acid, the term "plasmal" was used to describe these compounds present in the plasma

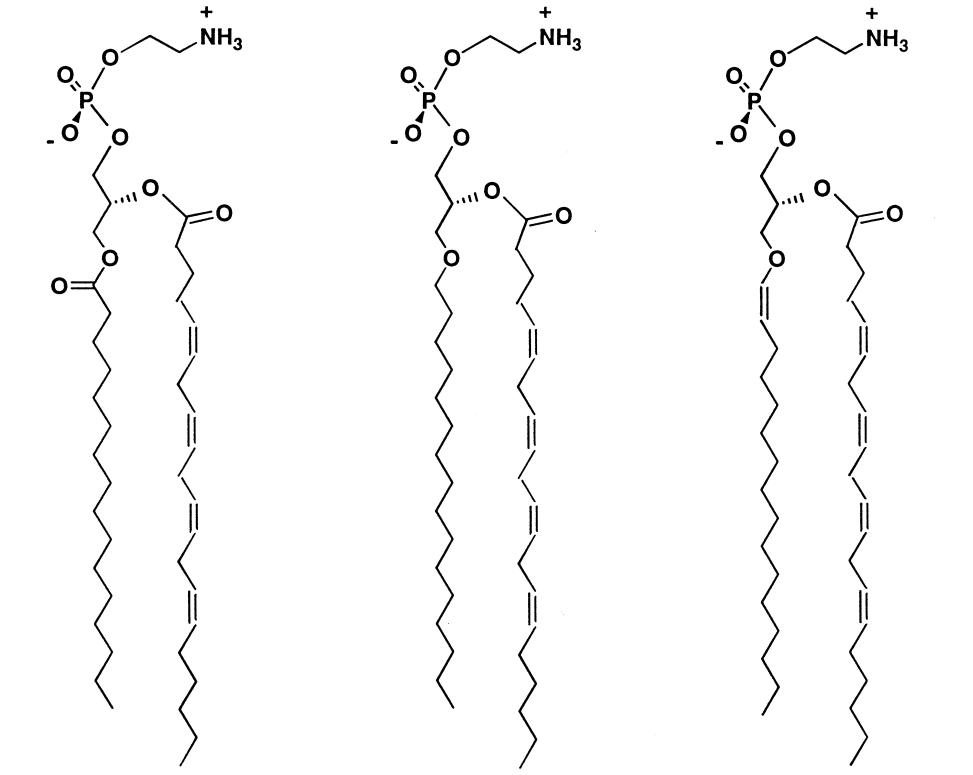

Fig. 1. The three sublasses of ethanolamine phospholipids.

(inside) of cells and the compound giving rise to these aldehydes was termed ''plasmalogen''. Although plasmalogens were initially thought to be diacetal phospholipids, work by Debuch [2] and Rapport et al. [3] identified the vinyl ether as the characteristic structural component, while work by Marinetti et al. [4] placed the vinyl ether bond at the *sn*-1 position.

Mass-wise, plasmalogens are the more abundant form of ether-linked phospholipids. In most tissues they exist primarily as members of the ethanolamine phospholipids (1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine; plasmenylethanolamine), where they can constitute up to 70% of that head group class [5]. Although choline-bearing ether phospholipids usually display the saturated ether linkage (1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; plasmanylcholine), certain tissues or cells contain significant levels of the plasmalogen form (1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine; plasmenylcholine). Ethanolamine and choline-linked headgroups make up the major portion of the plasmalogens found in animal cells, however minor amounts ($<0.2\%$ of total phospholipid mass) of serine and inositol-linked ether-phospholipids can also be found.

The nomenclature for plasmalogens varies. Many publications refer to the plasmalogen form of a phospholipid as the alkenylacyl form versus the alkylacyl (saturated ether linkage at the *sn*-1 position) or the diacyl forms. Therefore, the three subclasses within the ethanolamine phospholipids would be diacyl-GPE (1,2-diacyl-*sn*-glycero-3-phosphoethanolamine), alkylacyl-GPE (1-O-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine) and alkenylacyl-GPE (1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine).

For this review, like a good deal of the literature, the plasmalogen form of any head group class bears the pretext ''plasmenyl''. For example, the ethanolamine plasmalogen is called ''plasmenylethanolamine'' while ''plasmenylcholine'' is used for the choline plasmalogen. The phospholipids bearing the saturated ether linkage (1-O-alkyl) bear the pretext ''plasmanyl''. Therefore, a choline phospholipid, bearing this saturated ether is called plasmanylcholine. Finally, the diacylated species will be referred to as phosphatidyl (e.g. phosphatidylethanolamine and phosphatidylethanolamine). A number of abbreviations exist throughout the literature, however we will stay with these terms throughout this review.

3. Tissue distribution

Plasmalogens are widely distributed in the animal kingdom as well as in certain anaerobic microorganisms. Earlier reviews [5–7] have described the contents and compositions of naturally occurring ether phospholipids, including plasmalogens, in a variety of animal and human tissues.

Plasmalogens make up approximately 18% of the phospholipid mass in humans, however, the plasmalogen content of individual tissues or cell types varies widely. In general, the bulk of the plasmalogen pool is represented within the ethanolamine phospholipids where plasmenylethanolamine represents from 0 to 70% of this class. Liver contains very little plasmalogen; less than 5% of the ethanolamine phospholipids are the plasmalogen form. It does, however, export plasmenylethanolamine with lipoproteins. Vance [8] showed that, although there was little cellular plasmalogen, rat hepatocytes, in culture, secrete nascent lipoprotein in which 20–30% of the ethanolamine phospholipid was plasmenylethanolamine. Also, analysis of human serum [9] reveals significant levels of plasmenylethanolamine. These data suggest that liver cells target plasmenylethanolamine for export with lipoproteins.

Other tissues examined have significantly more plasmalogen. In erythrocytes, kidney, lung, testes and skeletal muscle, plasmenylethanolamine makes up 20–40% of ethanolamine phospholipids. The values are higher for brain, heart, lymphocytes, spleen, macrophages and polymorphonuclear leukocytes. We will discuss mammalian tissues containing relatively high levels of plasmalogens and have been used to examine plasmalogen function and biosynthesis.

3.1. Nervous tissue

Plasmalogens comprise a major portion of the phospholipids in the adult human central nervous system, however newborn levels are relatively low (7% of total phospholipid mass). This changes during early development. Balakrishnan et al. [10] reported an eight-fold increase in plasmenylethanolamine levels (per gram of tissue) in human brain white matter in the first year of life, so that plasmalogen (primarily plasmenylethanolamine) constituted approximately 20% of the phospholipid mass (70% of the ethanolamine phospholipids). In chicks, there is a significant increase in plasmalogens in the synaptisomal fractions during the first 3 days following hatching [11].

Between one-half to two thirds of the ethanolamine phospholipids in the whole brain are of the plasmalogen subclass and 11–12% of myelin phospholipid is plasmalogen. In 1977, Hack and Helmy [12] reported abundance, in myelin, of what was thought to be a plasmalogen species unique to myelin. This species, PI-PE-2, contained 18:1 as the principle hydrocarbon chain in both the 1 and 2 positions of the glycerol moiety. Boggs and Rangaraj [13] subsequently demonstrated an increase in the proportions of this plasmalogen during normal human development up to 17 years of age. This increase in 18:1-bearing plasmalogen was accompanied by a general increase in 18:1 in all glycerophospholipids in developing brain [14] possibly due to increased production or supply of this fatty acid.

3.2. Inflammatory and immunological cells

The spleen, the source for immunological cells such as macrophages and neutrophils, is enriched in plasmalogens; plasmenylethanolamine accounts for 66 and 50% of the total ethanolamine phospholipids in spleen [15] and human peripheral blood lymphocytes [16], respectively. Human polymorphonuclear leukocytes (neutrophils) are also enriched in plasmenylethanolamine. MacDonald and Sprecher [17] reported that 64–76% of ethanolamine phospholipids in human neutrophils were the plasmalogen subclass; other reports give values ranging from 33–66% depending upon the source and animal species [17–21]. The plasmenyl form makes up 40–60% of the ethanolamine phospholipids in macrophages and lymphocytes [22–24]. Values for macrophages from human have not been reported, however, when a human premyelocytic leukemic cell line, HL60, was stimulated to differentiate into a macrophage/monocyte lineage using phorbol ester, plasmalogen content increased [25].

Macrophages, neutrophils and leukocytes are unique in that they also contain significant levels of the saturated ether phospholipid, plasmanylcholine; up to 46% of the choline phospholipids are in the plasmanyl form in neutrophils [18–20] while the values were somewhat lower (approximately 30%) in macrophages and lymphocytes. This phospholipid is used by these cells for the formation of platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) [26].

Of particular note is the abundance of arachidonate associated with ether glycerophospholipids, compared to the diacyl subclass, in these cells. Macrophages and neutrophils contain high levels of arachidonic acid in the ethanolamine phospholipids, accounting for up to 40% of the fatty acid found at the *sn*-2 position [24,27]. In human neutrophils approximately 75% of the arachidonate associated with ethanolamine phospholipids are found in plasmenylethanolamine [27]. Similarly, in mouse [28] and rabbit [7,24] macrophages, twice as much of the plasmenylethanolamine bears arachidonate at the *sn*-2 position as the diacyl species. In contrast, one study by Mueller et al. [19], using rabbit neutrophils, showed little difference between the headgroup subclasses. However, in this latter case, little arachidonate was found in phospholipids compared to the other studies; arachidonate constituted only 4.5–6.3% of the fatty acid at the *sn*-2 position in any of the ethanolamine subclasses (inositol phospholipid analyses were not reported).

3.3. Cardiac tissue

Both skeletal muscle and cardiac tissue contain relatively high levels of plasmenylethanolamine [29–31]. Unique to cardiac tissue is the fact that the plasmenyl species makes up a large portion of the choline phospholipids. In human heart, 35–41% of the choline phospholipids are plasmenylcholine [32,33]. With 43–59% of the ethanolamine phospholipids also in the plasmenyl form in this organ, approximately one in every three phospholipids in the human heart is a plasmalogen. This also applies to bovine, rabbit, sheep and guinea pig heart [5]. In canine myocardial sarcolemma, 57% of the choline and 64% of the ethanolamine phospholipids were plasmalogen [34]. Again, arachidonate was high in plasmenylethanolamine; 75% of this phospholipid contained arachidonate. These findings have not been reported in human myocardial sarcolemma, although it seems reasonable to speculate a similar composition would be observed.

4. Biosynthesis

4.1. Plasmenylethanolamine biosynthesis

4.1.1. Dihydroxyacetone phosphate acyltransferase (step 1)

The biosynthetic pathway for ethanolamine plasmalogens is outlined in Fig. 2. We discuss its biosynthesis, first, since it is thought to be the precursor for plasmenylcholine. The first two reactions in the pathway take place in the peroxisomes. Plasmenylethanolamine biosynthesis begins with the esterification of the free hydroxyl group of dihydroxyacetone phosphate (DHAP) with a molecule of long chain ($>$C10) acyl CoA, catalyzed by dihydroxyacetone phosphate acyltransferase (glycerone-3-phosphate acyltransferase; EC 2.3.1.42), abbreviated as DHAPAT. The activity has been purified from guinea pig liver as a single, hydrophobic polypeptide with an approximate molecular weight of 70 kDa [35]. The peroxisomal enzyme is unique in that it displays activity over a rather broad pH range with optimal activity at approximately pH 5.5 [36,37]. It is also resistant to inhibition by the sulfhydryl-binding reagent N-ethylmaleimide (NEM) and will utilize only DHAP as the acyl acceptor. These characteristics distinguish it from a second DHAPAT activity localized in the microsomes.

Schlossman and Bell [38,39] reported a second DHAPAT activity in microsomes from murine tissues and suggested that this DHAPAT activity was a dual function of the microsomal glycerol-3-

phosphate acyltransferase (G3PAT; EC 2.3.1.15). Both microsomal G3PAT and DHAPAT activities displayed the same pH optimum and selectivity toward acyl-CoA substrates as well as similar sensitivities toward heat inactivation, NEM, trypsin and other conditions. Both G3P and DHAP were competitive inhibitors for the other substrate. Coleman and Bell [40] reported an increase in NEM-sensitive DHAPAT in concert with an increase in microsomal G3PAT during differentiation of 3T3-L1 cells into adipocytes. In contrast to these studies, Datta and Hajra [41] presented data, using guinea pig liver, supportive of a single NEM-resistant, peroxisomal DHAPAT activity.

The questions concerning the existence of a second DHAPAT outside of the peroxisome and the ability of the microsomal G3PAT to acylate DHAP have been difficult to answer due to concerns about cross-contamination of sub-cellular fractions and assay conditions. However, evidence using somatic cell mutants supports the notion of a second DHAPAT. Mutants, isolated from rodent cell lines, defective in peroxisomal DHAPAT activity, lost almost all activity when measured at pH 5.5; however, they maintained substantial activity at pH 7.4 [42–44]. This residual DHAPAT activity was NEM-sensitive [43,44], similar to G3PAT. Transfection of these mutants with human peroxisomal DHAPAT resulted in seven-fold greater pDHAPAT than wild-type cells, yet the levels of NEM-sensitive activity remained unaffected (Liu, D. and Zoeller, R.A., unpublished data). These findings suggest the presence of another, independent DHAPAT activity. Whether this second, NEM-sensitive DHAPAT activity is due to the microsomal G3PAT is unclear since neither activity has been purified.

Thai et al. [45] reported the cloning and sequencing of human, peroxisomal DHAPAT using the sequence information obtained from the enzyme partially purified from rabbit Harderian

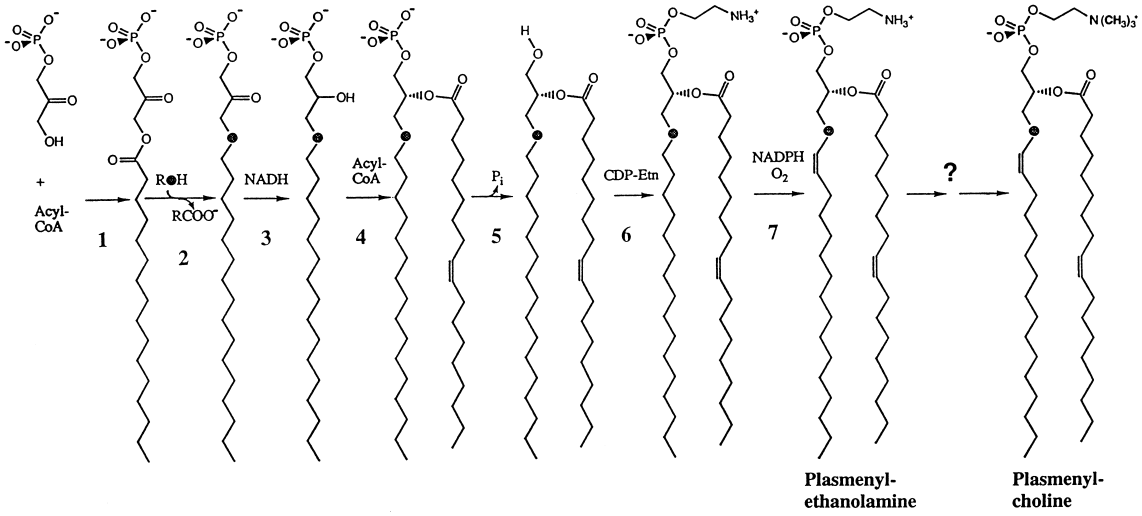

Fig. 2. Plasmalogen biosynthesis in animal cells. Note that the oxygen from the fatty alcohol (darkened in the figure) comes from the fatty alcohol in step 3. Also note that plasmanylethanolamine is the immediate precursor for plasmenylethanolamine. Step 1; dihydroxyacetone phosphate acyltransferase; step 2; Alkyl-dihydroxyacetone phosphate synthase; step 3; Acyl/alkyl-dihydroxyacetone phosphate reductase; step 4; Lysophosphatidate acyltransferase; step 5; phosphatidate phosphohydrolase I; step 6; CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase; step 7; plasmanylethanolamine desaturase.

gland peroxisomes. Sucrose gradient centrifugation of the CHAPS extracted peroxisomes resulted in a trimeric complex of 76 KDa, 72 KDa and 69 KDa, which co-purified with the DHAPAT activity. Protein sequencing revealed that the 76 KDa protein was alkyl-DHAP synthase, whereas both the 72 KDa and 69 KDa proteins corresponded to DHAPAT. The authors suggest that the 69 KDa polypeptide is most likely a modified form of DHAPAT, presumably generated by a post-translational modification which still needs to be characterized.

The matching sequences obtained from the identified EST-clones as well as clones obtained from a human brain cDNA library screen revealed the complete nucleotide sequence of the human DHAPAT cDNA. The corresponding human DHAPAT protein sequence revealed a protein consisting of 680 amino acids exhibiting a molecular mass of 77,187 Da. Most notable was the presence of a C-terminal Peroxisomal Targeting Signal 1 (PTS1) sequence represented by a tripeptide AKL that has been found in various other peroxisomal matrix proteins [46]. Immunocytochemical localization of DHAPAT to the inner aspect of the rat liver peroxisomal membrane confirmed this finding. Ofman et al. [47] also reported the cloning and sequencing of the human cDNA for DHAPAT containing an identical ORF sequence information encoding a 77.2 KDa protein product. Again, this sequence contained a PTS1 targeting sequence. The cDNA sequence for murine DHAPAT has been reported [48]. The murine cDNA coded for a 678 AA protein displaying 80.5% similarity to the human protein.

Screening of protein databases revealed homologies of human DHAPAT with glycerol-3-phosphate acyltransferase (G3PAT) from a wide species variety, including rat and mouse mitochondria, *E. coli* and *Haemophilus influenzae*. The homologies are restricted to distinct domains of about 35–80 amino acid residues in length showing up to 78% similarity and up to 45% identity.

The peroxisomal DHAPAT activity is crucial for plasmalogen biosynthesis. DHAPAT$^-$ mutants and human fibroblasts lacking this activity are unable to synthesize plasmenylethanolamine (the primary plasmalogen species), while diacyl-phospholipid synthesis remain unaffected. In fact, the rate of phosphatidylethanolamine biosynthesis and its levels are increased to compensate for this loss [42,44]. Thus, cellular ethanolamine phospholipid mass is maintained. Although loss of peroxisomal DHAPAT activity does not affect the synthesis of non-ether-linked glycerolipids, evidence presented by Hajra et al. [49] demonstrated that increases in this enzyme could participate in the increased synthesis of triacylglycerides; during conversion of the preadipocyte cell line 3T3-L1 to adipocytes, peroxisomal DHAPAT activity is induced nine-fold.

4.1.2. Alkyl-DHAP synthase (step 2)

The next reaction results in the formation of the ether bond by replacement of the *sn*-1 fatty acid with a long chain fatty alcohol. This unique biochemical reaction is catalyzed by alkyl-DHAP synthase, EC 2.5.1.26 [50], also an exclusively peroxisomal enzyme. The activity has been purified to homogeneity as a single polypeptide of approximately 65 kDa on SDS-PAGE [51]. This molecular weight agreed with the cDNA sequence obtained through PCR amplification [52]. The synthase differs from DHAPAT in that it does not appear to be an integral membrane protein; analysis of its amino acid sequence reveals no hydrophobic regions that would suggest transmembrane domains and the purified enzyme is partly soluble. Also, the nascent synthase bears a N-terminal peroxisomal targeting signal sequence, PTS2, which is proteolytically cleaved.

Evidence suggests that DHAPAT and the synthase are tightly associated within the cells. Both activities can be localized to the luminal side of the peroxisome [53]. Bierman et al. [54] demonstrated

that, following treatment of cells with cross-linking reagents, DHAPAT could be immunoprecipitated in a 210 kDa heterotrimeric complex with the synthase. Also, there is evidence that DHAPAT activity is dependent upon this physical association for proper activity. DHAPAT and alkyl-DHAP synthase are targeted to the peroxisome using different targeting sequences. The synthase is targeted to the peroxisome using the PTS2 and human fibroblasts with defects in this targeting system (e.g. in RCDP) are deficient in this activity, however these cells are also deficient in DHAPAT, which uses the PTS1 system for targeting. Also, de Vet et al. [55] have shown that human fibroblasts, with mutations in the alkyl-DHAP synthase gene that resulted in early termination, resulted in a DHAPAT$^-$ phenotype, even though the DHAPAT gene, itself, is unaffected. The finding by Hajra et al. [49] that peroxisomal DHAPAT activity increases nine-fold in the differentiating 3T3-L1 cell while the synthase activity actually decreases is not consistent with this hypothesis, although sufficient quantities of the synthase polypeptide may still be present to support this increase in activity.

4.1.3. Acyl/alkyl-DHAP reductase (step 3)

The third step in plasmalogen biosynthesis, catalyzed by acyl/alkyl-DHAP reductase (1-palmitoyl-*sn*-glycero-3-phosphate: NADP$^+$ oxidoreductase; EC 1.1.1.101) , is the fusion point between ether lipid biosynthesis (including plasmalogens) and the synthesis of diacyl-phospholipids. The synthase, which reduces the ketone function at the *sn*-2 position of 1-alkyl-DHAP, forms 1-alkyl-2-lyso-*sn*-glycero-3-phosphate, the ether-linked analogue of lysophosphatidate (1-acyl-2-lyso-*sn*-glycero-3-phosphate). The reduction is achieved through a classical hydride transfer mechanism involving NADPH as the co-factor. Purification of the activity approximately 5500-fold from pig liver peroxisomes resulted in a single polypeptide of 60 kDa [56]. The protein contained 27% hydrophobic amino acids and required the use of strong detergents to solubilize the activity.

Evidence suggests that this enzyme is utilized in the synthesis of both ether lipids (including plasmalogens) and diacylated phospholipids. The purified reductase is able to reduce either alkyl-DHAP or acyl-DHAP as substrate with similar Km and Vmax values for either substrate [56]. Also, a mutant cell line, deficient in this activity, demonstrated a 56% decreased plasmenylethanolamine biosynthesis as well as a 57% reduction in synthesis of phosphatidylcholine [57]. These latter results suggest that the acylation of, not only G3P, but also DHAP can be quantitatively important for diacyl-phospholipid biosynthesis. These finding are consistent with data provided by Hajra et al. [49]. Using dual-isotope labeling of 3T3-L1 cells, with *D*-[3-^{3}H, U-^{14}C]glucose, they estimated that 36 and 19% of newly formed phosphatidylcholine and phosphatidylethanolamine, respectively, were synthesized using acyl-DHAP as an intermediate in the pathway.

Since the reductase activity is important for both pathways, it makes sense that it would be localized to the peroxisome (where alkyl-DHAP is synthesized) and the endoplasmic reticulum where acyl-DHAP can be produced by the putative microsomal DHAPAT discussed above. Dual localization of this enzyme has been documented [58,59], however, location appears to be less of a priority for this enzyme's activity; both human and rodent cells defective in peroxisome assembly, display normal levels of acyl/alkyl-DHAP reductase activity, despite a complete loss of the exclusively peroxisomal activities, DHAPAT and alkyl-DHAP-synthase [43,60,61].

4.1.4. Lysophosphatidate acyltransferase (step 4)

The next three reactions outlined in Fig. 2 are all catalyzed by microsomal enzyme systems and all these steps are utilized for the synthesis of both diacyl-phospholipid and ether lipids, resulting

in the formation of either phosphatidylethanolamine or plasmanylethanolamine, depending upon whether lysophosphatidate or its ether-linked analogue are the starting material.

Lysophosphatidate acyltransferases (LPA-AT), EC 2.3.1.51, catalyzes step 4. cDNA coding for the human and murine enzymes have been identified using sequence homologies to bacterial and yeast LPA-AT [62–66]. At least two independent human isozymes, alpha and beta, have been identified [66]. The alpha form was found in all tissues with enrichment in skeletal muscle while the beta form was found primarily in heart and liver. Stamps et al. [64] identified several splice variants and suggested that this enzyme may be regulated through alternative splicing. A polypeptide expressing this activity has not yet been purified to homogeneity from a mammalian source and specificities with respect to the *sn*-1 position on the glycerol backbone (ether vs. ester) are not known.

4.1.5. Phosphatidate phosphohydrolase I (step 5)

Phosphatidate phosphohydrolase I (PAP-I), EC 3.1.3.4, is responsible for removal of the phosphate. Again, it likely utilizes either diacyl-phosphatidate or its ether-linked analogue. This enzyme has not been purified and a mammalian cDNA has not been identified as yet, so it is impossible to say if there is an isozyme specific to plasmalogen biosynthesis.

4.1.6. CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase (step 6)

CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.1), EPT, catalyzes the attachment of the phosphoethanolamine head group (step 6). This activity has recently been purified to homogeneity from bovine liver [67]. The purified protein has an approximate molecular weight of 38 kDa, which corresponds well with the predicted size based upon the human cDNA that codes for this activity [68]. There is some question concerning the number of EPTs. Data from both the purified enzyme and the human cDNA definitively demonstrated that EPT contains a second, choline phosphotransferase activity as well, suggesting that this protein is involved in the synthesis of both ethanolamine and choline phospholipids. However, an animal cell mutant, deficient in EPT activity, displayed normal CPT activity and phosphatidylcholine biosynthesis [69].

The purified EPT has not been tested for substrate specificity with respect to the *sn*-1 linkage, however Ford et al. [70] reported that the EPT activity associated with rabbit myocardium displayed a selectivity for diradylglycerols substrates with an alkenyl linkage at the *sn*-1 position. It is unclear how this would relate to de novo biosynthesis of plasmenylethanolamine since the substrate for EPT would be the *sn*-1-alkyl form (Fig. 2). This may well represent a scavenging mechanism. There is no compelling evidence, to date, that any of the enzymes catalyzing steps 4 through 6 have specificity for the 1-alkyl over the 1-acyl substrate.

4.1.7. Plasmanylethanolamine desaturase (step 7)

The final step in the biosynthetic pathway for plasmenylethanolamine involves the insertion of a double bond between the C1 and C2 of the alkyl chain of plasmanylethanolamine forming the characteristic "vinyl-ether" linkage found in plasmalogens. It is catalyzed by the enzyme plasmanylethanolamine desaturase ($\Delta 1'$-desaturase, EC 1.14.99.19). Although not well characterized, the $\Delta 1'$-desaturase appears to be similar to some of the fatty acyl-CoA desaturases [71,72] in that it is a membrane-bound, multicomponent system consisting of an electron transport system

(cytochrome b_5 and cytochrome b_5 reductase) and a terminal, cyanide-sensitive desaturase protein that is responsible for substrate specificity. The terminal desaturase has not been isolated and there had been some speculation that $\Delta 1'$-desaturation is an alternate activity of one of the fatty acyl-CoA desaturases. This seems unlikely, however; Lee et al. [73] demonstrated that the $\Delta 1'$-desaturase and the stearoyl-CoA desaturase activities are differentially regulated by dietary regimens. Also, a mutant cell line, deficient in the $\Delta 1'$-desaturase activity, was normal with respect to the conversion of stearate and linoleate to oleate and arachidonate, respectively [74].

4.1.8. Possible scavenging pathway: alkylglycerol kinase

Plasmalogens may also be formed from alkylglycerols, bypassing the first three steps through the action of a kinase, ATP: 1-alkyl-*sn*-glycerol phosphotransferase (EC 2.7.1.93, alkylglycerol kinase). The product, l-O-alkyl-2-lyso-*sn*-glycero-3-phosphate enters the pathway after the reductase step. This activity has never been purified and it is not certain whether it can use monoacylglycerols as substrate [75].

This pathway can contribute significantly towards plasmalogen biosynthesis. For example, in dietary studies, when young rats were fed 1-O-heptadecyl-*rac*-glycerol, up to 62% of the alkenyl groups in the tissues were 17:0 [76]. Also, alkylglycerol supplementation of mutant cells, defective in the early steps in plasmalogen biosynthesis resulted in full recovery of plasmalogen content [77–79]. It is possible that this represents a salvage pathway of sorts following partial enzyme-catalyzed degradation of plasmalogens; although we do not know if the alkylglycerol kinase can utilize alkenylglycerols as substrates, supplementation of plasmalogen-deficient cells with alkenylglycerols also restored plasmalogen levels [79].

4.1.9. Alternative pathways for head-group placement

Ethanolamine phospholipids can also be synthesized through headgroup transfer using phosphatidylserine or phosphatidylcholine, or through the decarboxylation of phosphatidylserine [80–82] , however these pathways are likely more important for the synthesis of the diacyl species, phosphatidylethanolamine. The biosynthesis of plasmenylethanolamine appears to rely heavily, upon the pathway described in Fig. 2. In CHO mutants defective in EPT [69] the synthesis of plasmenylethanolamine was severely reduced, while phosphatidylethanolamine synthesis remained relatively unaffected.

4.2. Origins of the fatty alcohol

Fatty alcohol is required for plasmalogen biosynthesis as a substrate for the alkyl-DHAP synthase (step 2). Three possibilities have been considered as to the origin of the fatty alcohol moiety utilized for plasmalogen biosynthesis, although the quantitative contribution of each is as yet unclear. The first possibility is dietary. Dietary fatty alcohols would come primarily initially in the form of wax ester found in certain vegetables and fish. Long-chain fatty alcohols are readily taken up by cells, probably through passive diffusion across the membranes, and can be incorporated into plasmalogens [83,84].

In the absence of an exogenous source, fatty alcohols can be formed from fatty acids by the reduction of fatty-acyl-CoA [85]. This reaction is catalyzed by long-chain fatty acyl-CoA reductase, which uses NADPH as a cofactor [86]. This activity appears to be responsive to cellular plasmalogen levels; Rizzo et al. [87] demonstrated that fibroblasts from patients with plasmalogen-deficiency displayed a

two–seven-fold increase in the rate of hexadecanol synthesis due to an increase in the activity of acyl-CoA-reductase.

More recently, Hayashi and Hara [88] have proposed that long-chain fatty alcohols, used for plasmalogen biosynthesis, are produced primarily within the peroxisomes during peroxisomal β-oxidation. Rats, injected with [1-^{14}C]hexadecanol or [1-^{14}C]hexadecanoic acid incorporated label into only diacyl phospholipids in the liver while radioactivity from [1-^{14}C]lignoceric acid (24:0; oxidized by the peroxisomal β-oxidation system) appeared in the alkenyl portion of plasmalogens, primarily as 16 and 18 carbon alkenyl chains. They hypothesized that ^{14}C-acetate, as acetyl-CoA, is produced from lignoceric acid as a by-product of peroxisomal-β-oxidation and then incorporated during the NADPH-dependent chain elongation of dodecanoyl-CoA. Ultimately, this resulted in labeled hexadecanol, which would now be available for incorporation into plasmalogens.

4.3. Plasmenylcholine biosynthesis

Although the pathway for plasmenylethanolamine has been fairly well characterized, the mechanism for plasmenylcholine biosynthesis is still an issue. Plasmenylcholine biosynthesis does not appear to be analogous to that of plasmenylethanolamine; the Δ1′-desaturase does not effectively utilize plasmanylcholine or lysoplasmanylcholine as a substrate [89,90] and rabbit heart was capable of rapidly converting [1′,2′-alkyl-$^{3}H_2$]1-O-alkyl-2-lyso-*sn*-glycero-3-phosphocholine to labeled plasmanylcholine, but not to plasmenylcholine [91].

All evidence supports the notion that plasmenylethanolamine is the precursor for plasmenylcholine [92,93], although the mechanism for this process has not been elucidated. Most of the evidence points to some form of polar head-group transfer or remodeling. Remodeling can occur via several possible pathways. The simplest would be a headgroup transfer, using plasmenylethanolamine, analogous to the synthesis of phosphatidylserine in animal cells [81] as represented in Scheme 1. Such a reaction would require a headgroup transferase specific for plasmenylethanolamine that can facilitate an exchange of a choline base group for the ethanolamine moiety of plasmenylethanolamine, however, no reports describing this activity have been produced.

Most evidence points to the use of alkenylglycerol (obtained from plasmenylethanolamine) and CDP-choline to form plasmenylcholine (Scheme 2). Possibly the most compelling evidence for this pathway is the accumulation alkenylglycerols in rabbit myocardial membranes [93] as well as the guinea pig heart [94]; both are rich in plasmenylcholine.

How alkenylacyl-glycerol is produced is still not clear. The simplest explanation would be the existence of a plasmenylethanolamine-specific phospholipase C, not present in other tissues. Wolf and Gross [95] reported the partial purification of a phospholipase C in canine myocardium that is specific for choline and ethanolamine phospholipids and is capable of hydrolyzing plasmenylethanolamine to 1-alkenyl-2-acyl-*sn*-glycerol. However, no preference for the plasmenyl form

Scheme 1.

Plasmenylethanolamine + choline ⇄ **plasmenylcholine + ethanolamine**

Scheme 1.

Scheme 2.

Plasmenylethanolamine $\xrightarrow{\text{PLC}}$ **1-alkenyl-2-acyl-*sn*-glycerol + phosphoethanolamine**

1-alkenyl-2-acyl-sn-glycerol + CDP-choline $\xrightarrow{\text{CPT}}$ **plasmenylcholine**

Abbreviations: PLC, phospholipase C; CPT, CDP-choline: diacylglycerol ethanolaminephosphotransferase.

Scheme 2.

Scheme 3.

1-alkenyl-2-acyl-GPE $\xrightarrow{\text{PLA}_2}$ **1-alkenyl-2-lyso-GPE + fatty acid**

1-alkenyl-2-lyso-GPE $\xrightarrow{\text{Lvso-PLC}}$ **1-alkenyl-glycerol + phosphoethanolamine**

1-alkenyl-glycerol $\xrightarrow{\text{AEG Kinase}}$ **1-alkenyl-2-lyso-glycero-3-phosphate (G3P)**

1-alkenyl-2-lyso-G3P + acyl CoA $\xrightarrow{\text{FA-CoA AT}}$ **1-alkenyl-2-acyl-G3P + CoASH**

1-alkenyl-2-acyl-G3P $\xrightarrow{\text{PAP1}}$ **1-alkenyl-2-acyl-glycerol + phosphate**

1-alkenyl-2-acyl-G3P + CDP-choline $\xrightarrow{\text{CPT}}$ **plasmenylcholine + CMP**

Abbreviations: Lyso-PLC, lysophospholipase C; FA-CoA AT; Fatty acyl-Co acyltransferase; PAP1, phosphatidate phosphohydrolase 1; CPT, CDP-choline: diacylglycerol ethanolaminephosphotransferase; GPE, glycerol-3-phosphoethanolamine.

Scheme 3.

was demonstrated. Another mechanism for production of plasmenylcholine includes a series of both sn-2 position as well as polar-head group modifications as illustrated in Scheme 3.

This is initiated by the action of a phospholipase A_2 (PLA_2) followed by phospholipase C (PLC) activity on the resulting product. The alkenylglycerol would be phosphorylated and acylated to generate the plasmalogen form of phosphatidate. This could be dephosphorylated by PAP1 and the phosphocholine headgroup attached by the choline phosphotransferase (CPT). The possibility that phospholipase D was important in this process was eliminated by showing that MDCK cells labeled with the PLD-resistant 1-O-[^{3}H]alkenyl-2-acyl-*sn*-glycero-3-phosphonoethanolamine were able to synthesize the corresponding [^{3}H]-labeled choline plasmalogen [96]. Both this study and the study by Lee et al. [92] argued against S-adenosyl-methionine dependent methylation of plasmenylethanolamine as a major contributing pathway.

In summary, a clear description of the pathway for plasmenylcholine biosynthesis has not yet been identified. It is clear, however, that plasmenylethanolamine is an essential precursor to choline plasmalogens in all animal cell types tested so far. The pathways described in these schemes may each contribute to plasmenylcholine biosynthesis, depending upon factors such as the external cellular stimulus, and physiological state of the cell.

5. Regulation of plasmalogen levels

Our understanding of the mechanisms that regulate plasmalogen levels in different membranes/tissues is limited. Also, in talking about regulating plasmalogen biosynthesis, we must consider the regulation of plasmenylethanolamine and plasmenylcholine separately in certain tissues; many cell types, including most cultured cell lines, contain little or no plasmenylcholine. On the other hand, when studying tissues that contain significant levels of both species, regulation of the biosynthesis of each is likely interdependent.

5.1. Plasmenylethanolamine

A rate-limiting step in the biosynthesis of plasmenylethanolamine has not been identified. If there is a control point for plasmenylethanolamine biosynthesis it is likely downstream of the first three steps in the pathway. Two lines of evidence support this notion. First, attempts to elevate plasmenylethanolamine levels by supplementation with alkylglycerols (AG; glycerol with the ether-linked alkyl chain) have failed. As mentioned above (Section 4.1.8), AG are able to enter cells and enter the pathway as 1-O-alkyl-2-lyso-*sn*-glycero-3-phosphate, the product of the reductase (Fig. 2). Supplementation of human fibroblasts [97,98] and rodent cell lines [42,44,57,99], deficient in any of the first three enzymes, with AG restored normal levels of plasmenylethanolamine, but this did not increase levels significantly above normal; the addition of alkylglycerol did not influence plasmalogen levels in the normal or wild-type cell lines either [100]. In dietary studies, feeding rats alkylglycerol [76,101] failed to alter plasmalogen levels in all tissues examined even though the exogenous alkylglycerol was clearly entering the pathway; tissues in the test animals utilized the dietary compound for formation of a major portion of their plasmalogens [76]. Finally, transforming a DHAPAT$^-$ hamster cell line with a plasmid bearing human peroxisomal DHAPAT cDNA resulted in DHAPAT activity six times over wild-type cells

(CHO-K1), yet plasmalogen biosynthesis and levels were only elevated to wild-type values [102]; DHAPAT activity was not a crucial regulatory factor in determining plasmalogen levels.

Control of plasmenylethanolamine synthesis is likely to be complex due to the fact that the biosynthetic pathway is intertwined with the pathway for the synthesis of diacyl phospholipids, the two pathways sharing some of the same enzymatic machinery. Also, there is communication between these pathways. For example, in mutant cell lines defective in plasmenylethanolamine biosynthesis, there is a compensating increase in the biosynthetic rate and steady state levels of phosphatidylethanolamine, thus maintaining the phospholipid headgroup composition [43,76,99].

Although evidence points towards regulation downstream, the question of what regulates plasmenylethanolamine biosynthesis remains. It is unclear whether there is a differential regulation of the diacyl and plasmalogen classes of phospholipids through common biosynthetic enzymes or whether isozymes, with unique substrate specificities (e.g. acyl vs. alkyl) are differentially expressed and regulated. Since CDP-ethanolamine may react with diacylglycerols, alkylacylglycerols, or alkenylacylglycerols, the enzyme CDP-phosphoethanolamine phosphotransferase is at a crucial branch point, and may have different properties with these substrates during different phases of development. The apparent K_m of EPT for CDP-ethanolamine in the presence of alkylacylglycerols (AAG) or alkenylacyl glycerols (AEG) was about three times lower than in the presence of diacylglycerols (DAG) during the develop-ment of chick brain [103]. Such variations may arise from a differential expression of isoenzymes specific for plasmalogen biosynthesis in the brain during different stages of myelination and development.

5.2. Plasmenylcholine

Given the lack of knowledge concerning plasmenylcholine biosynthesis, it is difficult to speculate about the control of plasmenylcholine levels. Xu et al. [94] reported that the ratio of diacylglycerol to alkenylacylglycerol in guinea pig heart (42:1) was 10 times that observed in liver (422:1), an organ that contains relatively little plasmenylcholine, while Ford and Gross [93] reported a similar diacylglycerol:alkenylacylglycerol ratio (approximately 20:1) in the rabbit myocardial membranes. It was suggested that modulation of this ratio is responsible for regulating plasmenylcholine biosynthesis. The increased ratios, however do not account for the 2:1 ratio of phosphatidylcholine: plasmenylcholine observed in these tissues. Ford and Gross [93] suggested the preferential use of the alkenylacylglycerol by a cholinephosphotransferase to partially explain elevated plasmenylcholine levels under these conditions. To date, no alkenylacyl-specific CPT has been isolated.

6. Plasmalogen-deficient cells

6.1. Human plasmalogen-deficiency disorders

The fact that plasmalogens are important in physiological processes is supported by the description of a series of human genetic disorders in which the patients' tissues lack plasmalogens. In 1983, Heymans et al. [104] described plasmalogen deficiency in tissues from a patient suffering from an inborn error in peroxisome assembly. It was established that this was due to the loss of peroxisomal DHAPAT [105] and alkyl-DHAP synthase [106] activities. These enzymes, localized

to the peroxisomal membrane, catalyze the first 2 steps in plasmalogen biosynthesis (Section 4); peroxisome-deficient cells invariably lack these enzymes and are therefore plasmalogen-deficient. The patients suffer a variety of pathologies including severe mental retardation, hypotonicity, adrenal dysfunction, cataracts, deafness, facial dysmorphism, chondrodysplasia and a failure to thrive. Death occurs often within the first year of life.

A series of autosomal, recessive inherited disorders in which patient's tissues lack peroxisomes or peroxisomal function have been described and reviewed [107,108]. The disorders can be divided into three clinical entities; Groups A, B, and C disorders of peroxisome biogenesis. Group A disorders are associated with a generalized loss of peroxisomes and the correspondingly all or most peroxisomal functions. This is due to an absence or a severe reduction in the number of peroxisomes in the tissues. Zellweger syndrome and neonatal adrenoleukodystrophy (NALD) are well known examples of disorders belonging to this group.

Group B disorders involve the inability to properly target a subset of proteins to peroxisomes although many peroxisomal functions and the peroxisomal unit remain intact. An example of this group includes rhyzomelic condrodysplasia punctata (RCDP). First identified by Heymans et al. [109], RCDP is characterized by a disproportionately short stature, shortening of the proximal limb segments, facial dysmorphism, cataracts, severe mental retardation, and a high mortality rate. Patients affected with this disorder display a classical tetrad of biochemical abnormalities which includes: (1) and (2) defects in both DHAPAT and alkyl-DHAP synthase; (3) defective phytanic acid oxidation system due to a deficiency of phytanyl-CoA hydroxylase; and (4) a defective processing of 3-oxoacyl-CoA thiolase which is present in its immature 44 KDa molecular form. Since this form of RCDP was the first to be biochemically characterized, some articles in the literature report this as the type-1 disorder. Group B disorders are most commonly associated with a defect in the PEX7 gene that codes for the receptor of peroxisome targeting sequence 2 (PTS2) [110–112], thereby resulting in the loss of PTS2-dependent targeting of proteins to the peroxisome.

Group C disorders involve a loss of a single peroxisomal function or enzyme. A number of the cases involve a deficiency in one of the enzymes involved in plasmalogen biosynthesis. Some literature sources classify the disorders in group C as types 2 and 3 depending upon the deficiency of either DHAPAT or alkyl-DHAP synthase, respectively. In 1992, Wanders et al. [113] described a patient who displayed clinical symptoms consistent with RCDP, but lacked the classical tetrad of biochemical abnormalities. Instead, an isolated deficiency in DHAPAT was found (type 2 RCDP). Subsequently, a number of similar cases have been reported [114–118]. In 1994, Wanders et al. [119] reported another patient with an isolated defect in alkyl-DHAP synthase activity (type 3 RCDP).

The patients, in both Group A as well as Group B, display a variety of clinical pathologies, but the complex biochemical nature, loss of an organelle or multiple organelle functions, undermined our understanding the contribution that plasmalogen loss or the loss of ether lipids play in the pathology of the patients affected by these disorders. Patients with singular defects in plasmalogen biosynthesis commonly display many of the pathologies associated with the more global peroxisomal disorders including cranio-facial dysmorphisms, cataract formation, mental retardation, rhyzomelic shortening of the limbs and a lack of muscle tone. These findings demonstrate the importance of plasmalogens in normal development and/or functioning cellular function. Still, the biochemical links between the loss of plasmalogens and the pathologies associated with this has not been defined. A further struggle involves determining if the symptoms are associated

with the loss of plasmalogens in general, the loss of specific plasmalogen species or the loss of another ether-linked lipid such as plasmanylcholine (the precursor of platelet activating factor).

6.2. Mutant animal cell lines

A series of plasmalogen-deficient mutants, generated and isolated from established, immortal cell lines have been described. Basically, this involved random chemical mutagenesis of a population of cells followed by isolating the 1 in 10^4–10^6 cells that bear the phenotype desired, plasmalogen-deficiency. Table 1 lists the plasmalogen-deficient mutants isolated to date. Initial attempts at isolating plasmalogen-deficient mutants employed the Chinese hamster ovary cell line, CHO-K1, a fibroblast-like cell, as the parent strain [43]. This resulted in the isolation of ether-lipid deficient mutants, but the primary genetic lesion in these mutants was the loss of peroxisomes, similar to the Group A human disorders. Peroxisomal mutants have been instrumental in the identification of genes and factors involved in peroxisome assembly and function [120].

These mutants confirmed the central role of peroxisomes in plasmalogen biosynthesis and have served as useful somatic cell models for exploring inborn human disorders of peroxisome biogenesis such as the Zellweger syndrome. Their use in studying plasmalogen function was limited, however, due to the complications associated with the loss of an organelle and its functions. As with the human peroxisome-deficient fibroblasts, the addition of alkylglycerols to the growth medium, bypasses the enzymatic lesions, restoring plasmalogen levels to normal status. Reversal of altered phenotypes in alkylglycerol-supplemented cells indicates a relationship between plasmalogens and cell function. Still, the loss of this multifunctional organelle can complicate the interpretations of results.

When using the CHO cell line, the selection or screening techniques used to isolate plasmalogen-deficient mutants seemed to produce, almost exclusively, strains in which peroxisome-deficiency was the primary lesion [43,121]. In contrast, using the same selection protocols on the murine,

Table 1
Somatic cell lines with plasmalogen deficiencies

Mutant line	Phenotype	Enzymatic deficiency	Pathway step affected	Reference
CHO-K1 mutants				
ZR-78, 82, 87	Peroxisome deficiency 90% decrease in plasmalogen content	DHAPAT and Alkyl-DHAP synthase	Steps 1 and 2	[43]
NRel-4	90% decrease in plasmalogen content	DHAPAT	Step 1	[42]
NZel-1	90% decrease in plasmalogen content	Alkyl-DHAP synthase	Step 2	[99]
FAA.K1B	25% reduction in plasmalogen content 56% reduction in plasmalogen biosynthesis 46% reduction in total PL biosynthesis Accumulates alkylglycerols	Acyl/alkyl-DHAP reduction	Step 3	[57]
RAW 264.7 mutants				
RAW.108	90% decrease in plasmalogens	DHAPAT	Step 1	[44]
RAW.12	90% decrease in plasmalogen	DHAPAT and $\Delta 1'$-desaturase	Steps 1 and 7	[44]

macrophage-like cell line, RAW 264.7, generated primarily plasmalogen-deficient mutants that contained normal, functional peroxisomes; they displayed defects specific to plasmalogen-biosynthesis [44]. One of these mutants, RAW.108 was defective in the first step in plasmenylethanolamine biosynthesis, DHAPAT. The other, RAW.12 was deficient in both DHAPAT (step1) and the $\Delta 1'$-desaturase (step 7). Hybridization analyses showed that the DHAPAT deficiencies found in both RAW mutants were due to lesions in the same gene and that the desaturase lesion was genetically independent of the DHAPAT loss (R. A. Zoeller; unpublished data).

Later, using information obtained from the RAW mutants, new selection protocols were developed and used to obtain plasmalogen$^-$/peroxisome$^+$ mutants from a CHO-K1 background [42,44,99]. These new CHO mutants, NRel-4 and NZel-1, displayed singular defects in DHAPAT and alkyl-DHAP synthase, respectively. A third plasmalogen$^-$/peroxisome$^+$ CHO strain was isolated [57], FAA.K1B, that was defective in the third step in plasmenylethanolamine biosynthesis, alkyl-DHAP reductase.

6.3. Use of plasmalogen-deficient cells: advantages and problems

The availability of fibroblasts from patients with the plasmalogen-deficiency disorders as well as the somatic cell mutants have been extremely useful in confirming earlier work concerning plasmalogen biosynthesis and function as well as supplying new insights into these issues. With these cell lines, one is able to compare a plasmalogen-deficient strain with the parent or "normal" strain with respect to metabolism and a variety of cellular functions. In identifying alterations in a particular cellular function, one tentatively identifies a functional role for plasmalogens or ether lipids in that process. This relationship can be more firmly established by restoring plasmalogens to the cells through supplementation of the growth medium with AG. As explained in Section 4, this compound can enter cells, is phosphorylated and enters the biosynthetic pathway downstream of the first three steps. This is sufficient to completely recover plasmalogen levels [43,44,77,97].

Restoration of normal cellular function following AG supplementation places the plasmalogen/function relationship on firmer ground. In fact, comparisons made between mutant and AG-supplemented mutant are probably better comparisons than mutant vs. wild-type comparisons. This is because other genetic lesions, unrelated to plasmalogen biosynthesis, are generated by random chemical mutagenesis, and these can possibly be the underlying cause of the phenotype change. For example, on a number of occasions, a particular phenotype appeared in one plasmalogen-deficient CHO strain, yet it did not appear in additional, independently isolated plasmalogen-deficient strains. In these cases, the altered phenotype was not reversed upon AG supplementation. If AG supplementation does not reverse the phenotype to wild type-like, it is doubtful that plasmalogens or ether lipids are linked to that function.

AG supplementation restores other ether lipid species as well as plasmalogens. For instance, plasmanylcholine, a precursor for platelet activating factor, is restored upon AG supplementation. It is, therefore, difficult to identify plasmalogens, or the vinyl ether, as a crucial factor without additional supporting data. However, using one of the plasmalogen-deficient cell lines, RAW.12, the role of the vinyl ether can be tested. AG supplementation of this cell line does not restore plasmalogens. Although the DHAPAT lesion is bypassed, the $\Delta 1'$-desaturase deficiency in RAW.12 cells results in an accumulation of the 1-alkyl-2-acyl-phospholipid, plasmanylethanolamine (not the plasmalogen). Therefore, only the ether linkage is restored [44]. Plasmalogens can

be restored in this strain using *sn*-1-O-alkenylglycerol (AEG), which contains the vinyl ether group. This can also enter the biosynthetic pathway, bypassing both the DHAPAT and the desaturase lesions. Thus, one can titrate the restoration of either the ether bond with AG or the vinyl ether with AEG. Using this cell line, identification of the functions dependent upon the ether and the vinyl ether moieties have been identified [79,122].

One of the drawbacks in using the cell lines is that no established, immortal cell line has been identified that contains substantial levels of plasmenylcholine, the dominant plasmalogen species being plasmenylethanolamine. Therefore, studies on the synthesis and function of this subspecies are not possible in this system as yet. Identification of such a cell line would be of great help in this area.

7. Plasmalogen functions

In addition to serving as structural components of cellular membranes, a number of functions have been proposed for plasmalogens. These proposals are based upon their distribution into certain cell types, their physical and chemical properties, and changes in plasmalogen metabolism during the stimulation of certain cellular processes. Changes brought on by plasmalogen loss in mutant cells have also generated information concerning possible functions. We will discuss the more prevalent of these proposed functions and the evidence that support them.

7.1. Membrane dynamics

The loss of carbonyl oxygen and the presence of the vinyl ether double bond, give plasmalogens different physical properties when compared to their diacyl counterparts. Model membranes consisting of 100% semi-synthetic plasmenylethanolamine go through the transformation from lamellar gel to liquid-crystalline (Tm) at slightly lower temperatures (4–5°C) than the corresponding diacyl species [123]. More striking is the observation that the plasmenylethanolamine forms non-lamellar or non-bilayer structures (inverse hexagonal; Hex_{II}) at or below 30°C, while the diacyl analogues form these structures at much higher temperatures. These non-bilayer structures result in increased leakage of the membranes to ions and promotion of membrane-membrane fusions. This may have significance in certain cellular processes such as those that depend upon membrane fusions, including endocytosis and secretion. It is possible that plasmenylethanolamine is a key component in allowing the formation of the Hex II structures in biological membranes and is important in membrane fusion-mediated events. Phospholipid vesicles that contain 45–50% plasmenylethanolamine fused much more rapidly than those that contained the diacyl form [124]. The fact that plasmenylethanolamine is abundant in tissues and membrane systems that undergo rapid vesicular fusion events, such as synaptisomal membranes [11], would be consistent with this hypothesis.

Despite the data obtained from model systems, it has been difficult to obtain direct evidence that plasmalogens are required for the formation of non-bilayer structures or membrane-membrane fusions in the complex, heterogeneous biological membranes of the cell. Although present in very significant levels in some tissues, plasmenylethanolamine represents no greater that 15–20% of the total phospholipid mass [5]. Plasmenylcholine, the only other plasmalogen species to

exist in significant quantities, does not form the non-bilayer structures at physiological temperatures [123]. Given that phospholipids must share the membrane with cholesterol, glycolipids and proteins, it is unclear that local concentrations of plasmalogens, sufficient to induce membrane fusions, can be achieved. However, the fusion of biological membranes is certainly a multicomponent process and plasmenylethanolamine, in conjunction with other lipid or protein components, may be an important factor in fusion processes. Glaser and Gross [125] identified a protein in rat brain cytosol, glyceraldehyde-3-phosphate dehydrogenase, that was capable of facilitating fusion of plasmenylethanolamine-containing vesicles. Hermetter et al. [126] reported decreased membrane order in plasmalogen-deficient human fibroblasts. However, there were no significant changes in lipid mobility in vesicles prepared using extracted, phospholipids from these fibroblasts when compared to those prepared from plasmalogen-containing fibroblasts. The authors suggested that loss of plasmalogen interactions with other cellular components, possibly proteins, was responsible for the differences they observed in the intact cellular membranes.

There is evidence from plasmalogen-deficient cells that plasmalogens influence membrane dynamics within the cells. Mandel et al. [127] reported a significant decrease in HDL-mediated cholesterol efflux from the plasmalogen-deficient murine cell line, RAW.108, as well as from plasmalogen-deficient human fibroblasts. Restoration of plasmalogens through alkylglycerol supplementation restored cholesterol efflux to wild-type-like levels, demonstrating a direct relationship between the phenotype and plasmalogen loss. These data suggest a disruption of cholesterol transport or membrane trafficking in the absence of plasmalogens. The mechanistic reason for this phenotype is unclear. It is possible that vesicular transport of cholesterol, requiring membrane-membrane fusion, was affected. Alternatively, the ability of HDL to interact with the cell may have been altered. The importance of the ether bond and/or the vinyl ether are also unknown as yet.

7.2. Polyunsaturated fatty acids

One proposed function for plasmalogens is as sinks for polyunsaturated fatty acids in order to maintain high levels of these fatty acids in some tissues. A number of studies examining the fatty acid composition of plasmalogen phospholipids have demonstrated high levels of polyunsaturated fatty acids when compared with their diacyl counterparts [7,24,28,128,129]. Other studies have demonstrated that both n-6 and n-3 fatty acids are incorporated preferentially into plasmalogens [130–132]. Plasmalogens may serve as a preferred source of these fatty acids for their release during stimulation. This is particularly true for macrophages and neutrophils (see above), which have high levels of plasmalogens and respond to a variety of stimuli to form and release arachidonic acid.

Studies of patients with plasmalogen deficiency and the plasmalogen-deficient mutant cell lines do not support plasmalogens as being critical for maintaining high arachidonate levels in all tissues. From these studies, it seems as though plasmalogen status is more critical for maintaining n-3 polyunsaturated fatty acids (n-3 PUFA). In patients lacking plasmalogens, levels of docosahexaenoic acid (22:6n-3; DHA) are reduced in all tissues examined [133,134] as well as plasma and red blood cells [135], while arachidonic acid levels were significantly decreased only in plasma [135]. Neutrophils and macrophages, cell types noted for maintaining high arachidonate levels, were not examined from these patients; alterations in arachidonate content may be affected in

these cells. Plasmalogen-deficient RAW strains were deficient in only the n-3 PUFA [122]. This was accompanied by a 50% decrease in incorporation of ^{3}H-DHA into ethanolamine phospholipids (the only phospholipid class to contain significant levels of the plasmenyl form) over a 1.5-h labeling period, suggesting that DHA is inserted into plasmenylethanolamine during de novo synthesis or very shortly thereafter. In this case, the ether bond was the crucial element; recover of plasmanylethanolamine, which does not contain the vinyl ether, was sufficient for full recovery of n-3 PUFA. The vinyl ether double bond was not necessary for phenotype reversion.

7.3. Plasmalogens, arachidonate release and phospholipase A_2

Phospholipase A_2-mediated fatty acid release from phospholipid pools is stimulated by a variety of conditions including receptor/ligand binding and hypoxia or anoxia/reperfusion. There is significant evidence that plasmalogens may be an important source of arachidonate release during stimulation of cells. In rabbit smooth muscle cells 60% of the [^{3}H]arachidonic acid released from phospholipids following angiotensin II stimulation came from plasmenylethanolamine [129]. In A21387-stimulated human neutrophils, ether phospholipids, particularly plasmenylethanolamine, were a major source for released arachidonic acid [136,137]. Similar results were observed in A21387-stimulated mouse bone marrow-derived mast cells [138]. Thrombin stimulation of rabbit ventricular myocytes resulted in the formation of prostacyclin, concomitant with a loss of arachidonic acid from plasmenylethanolamine and plasmenylcholine; diacyl species were unaffected [139]. Cardiac tissue, which also contains high levels of arachidonate-bearing plasmalogens, released arachidonate in response to hypoxia or ischemia/reperfusion [140].

A number of studies have presented evidence for the activation of a plasmalogen-selective phospholipase A_2 (PLA_2) following cell stimulation. Hazen et al. [141] described the activation of a membrane-bound, plasmalogen-selective, PLA_2 in ischemic rabbit myocardium. Portilla et al. [142] reported a three-fold increase, in hypoxic rabbit proximal tubules, of a cytosolic PLA_2 with selectivity for plasmenylcholine. Similar results were reported using cells which had been exposed to interleukin 1β [143] or thrombin [139,144,145]. This activation coincided with a release of arachidonic acid and an accumulation of lysoplasmenylcholine [139,144,145]. Increases in both lysoplasmenylcholine an lysophosphatidylcholine have also been reported in hypoxic proximal tubules [146].

In 1985, Wolf and Gross described a plasmalogen-selective PLA_2 in canine myocardium [95]. Since then, three PLA_2s, with selectivity for plasmalogens have been isolated. The term "selective" is used because these enzymes, in vitro, display a preference for the plasmenyl form over the diacyl, although the diacyl form is also a substrate. The first, a 40 kDa protein [147] displayed a preference for plasmenylcholine over the plasmanyl and phosphatidyl forms and selectively utilized species bearing arachidonate at the *sn*-2 position. In 1996, Yang et al. [148,149] described a 39 kDa, plasmenylcholine-selective PLA_2, purified from bovine brain that displayed preference for plasmenylethanolamine over other ethanolamine phospholipids. Most recently, a cytosolic, 28 kDa protein was purified from rabbit kidney cortex [150]. The cDNA coding for this last PLA_2 have been isolated from both rat and rabbit and sequenced [151]. Northern analysis of various rat tissues showed expression in all tissues with the highest amounts of mRNA present in kidney and intestine. Since the cDNA sequence for only the kidney PLA_2 has been reported, the relationship between these proteins is unknown.

Unifying characteristics associated with the plasmalogen-selective PLA_2 activities is their lack of dependence on calcium and their sensitivity to inhibition by the plasmalogen analogue, (E)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one (bromoenol lactone; BEL) [152]. This compound displayed an IC_{50} of approximately 10^{-7} M for all three of the purified plasmalogen selective PLA_2s leaving other PLA_2s unaffected. Inhibition of cellular arachidonate release by BEL has been used as evidence to implicate a calcium-independent PLA_2 activity, possibly a plasmalogen-selective activity, in the release process [144,153,154]. However, the demonstration that BEL is also able to inhibit phosphatidate phosphohydrolase 1 (PAP1), an activity important for arachidonate release through a phospholipase D-mediated pathway [155], must also be considered when using this inhibitor.

In examining PLA_2-mediated arachidonate release in the plasmalogen-deficient RAW strains, we have been unable to chronicle any differences between wild type and mutant cells. Although arachidonate release following endotoxin and zymosan release are almost completely inhibited by BEL, similar amounts of [^{3}H]arachidonate were released with identical kinetics, in wild type and mutant strains. Alkylglycerol supplementation had no effect upon arachidonate release by the mutants (Gaposchkin and Zoeller, unpublished results).

7.4. Intracellular signaling

A number of studies link plasmalogens, or the generation of certain plasmalogen metabolites, to signaling cascades in response to stimuli. Most studies have focused upon the ether-linked diradylglycerols. Cells respond to a variety of stimuli to produce diacylglycerol from phospholipid pools. The diacylglycerols activate certain PKC isozymes, resulting in downstream signaling events. In some cases, alkylacyl- and alkenylacyl-glycerides are also formed. Daniel et al. [156] showed that Madin-Darby canine kidney cells, prelabeled with ether-linked 1-O-[3H]hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine, accumulated 1-O-[3H]hexadecyl-2-acyl-*sn*-glycerol when stimulated with phorbol ester. Subsequently Dougherty et al. [157] demonstrated increases in both diacylglycerols and 1-O-alkyl-2-acylglycerols in human polymorphonuclear leukocytes stimulated with fMet-Leu-Phe, phorbol ester, or A23187; the accumulation of the ether-linked diradylglycerol species was slower to develop and longer-lasting compared to diacylglycerol accumulation. The ether-linked diglyceride species resulted, possibly, from action of a lipase selective for ether phospholipids. At the same time, Ford and Gross [158] reported that 1-O-alk-1′-enyl-2-acyl-sn-glycerol accumulated in ischemic rabbit heart. More recently, Musial et al. [159] have shown the accumulation of ether-linked diglycerides in rat glomerular messangial cells stimulated with interleukin 1α; diacylglycerols did not accumulate under this treatment. Interestingly, this effect was specific for interleukin 1α; other stimuli induced diacylglycerol accumulation only.

It is clear that ether-linked diglycerides accumulate following stimuli, but what are they doing? Earlier studies demonstrated that, in vitro, synthetic ether-linked diglycerides do not activate PKC [160–163]; they may, in fact, be considered competitive inhibitors of diacylglycerides for PKC. This would suggest that plasmalogens, or their diradylglyceride products, are involved in regulating or attenuating certain PKC-mediated responses. However, the influence of these ether-linked diglycerides on PKC activity depends upon the specific PKC isoform and the assay conditions. Ford et al. [164] demonstrated a stimulation of specific PKC isoforms (e.g. PKC-β) by ether-linked diglycerides in the presence of free calcium concentrations greater than 1 μM, while

Mandal et al. [165] found significant inhibition of PKC isoforms δ and ε (calcium–independent isoforms) using ether-linked diglycerides in cell-free systems. In plasmalogen-deficient human fibroblasts, the restoration of plasmalogens results in inhibition of PKC-α translocation to the membranes and phospholipase D activation in response to bradykinin [166]; it did not affect PKC-ε bradykinin-induced MAP kinase activation.

Plasmalogen-derived products, other than the diradylglycerols, are also suspected signaling molecules. A lysoplasmenylcholine-responsive protein kinase isolated from cardiac cytosol was identified as cAMP-dependent protein kinase [167]. Ford and Hale [168] reported that the sodium–calcium exchanger displayed 10-times the activity in plasmalogen-containing liposomes compared to those containing diacyl phospholipids, while Hale et al. [169] reported a seven-fold increase when the plasmalogen form of phosphatidate was generated in proteoliposomes using phospholipase D. Plasmenyl lysophosphatidate has been identified as a possible growth-stimulating agent, similar to the acyl form [170,171], possibly having a separate receptor.

7.5. Plasmalogens as antioxidants

In 1972, Yavin and Gatt [172,173], attempting to isolate a plasmalogen-cleaving enzyme from rat brain homogenates, described the oxygen/iron-dependent cleavage of the vinyl ether linkage of plasmalogens by ascorbic acid; the physiological significance of this was not discussed at that time. In 1986, Marmer et al. [174], using ethyl hexadecyl-1-enyl ether as a plasmalogen model, described the oxygen-dependent breakdown of the vinyl ether. The rate of vinyl ether loss was markedly increased in the presence of polyunsaturated fatty acids. They concluded that the instability of the vinyl ether associated with plasmalogens may contribute to development the rancidity in meat upon storage. Similar results were reported using 1-O-alk-1′-enyl-2,3,-di-O-acylglycerols as model compounds [175]. In this light, plasmalogen appeared to be a possible target for oxidative damage and cells containing high levels of these phospholipids would be at greater risk to reactive oxygen-mediated damage. Studies using plasmalogen-deficient cells and in vitro model systems have recently supplied evidence that plasmalogens may, in fact, serve a protective role during oxidant-induced stress.

7.5.1. Cellular studies

In 1988, evidence emerged that plasmalogens may be protective against reactive oxygen species (ROS), functioning as endogenous antioxidants [78]. CHO cells were killed when exposed to a pyrene-labeled fatty acid, 12-(1′-pyrene)dodecanoic acid (P12), followed by irradiation with long-wavelength UV light (>300 nm). Cytotoxicity was due to the generation of singlet oxygen and/or other ROS arising from the excitation of the pyrene moiety (photosensitizer) by the UV light. Interestingly, peroxisome/plasmalogen-deficient variants of this cell line were much more sensitive to this treatment. This hypersensitivity could, in part, be explained by the fact that the mutant cells accumulated more P12 that the parent strain; the accumulation was apparently due to the loss of the peroxisomal β-oxidation system [176]. However, the loss of plasmalogens appeared to be a factor as well; restoration of plasmalogens to the mutants through AG supplementation partially rescued these cells during P12/UV exposure. In an accompanying paper, Morand et al. [177] demonstrated the selective decomposition of plasmalogens in membranes of wild-type CHO cells subjected to P12/UV and the generation of products consistent with the ROS-mediated breakdown of plasmenylethanolamine. Similar observations concerning P12/UV hypersensitivity

were made using peroxisome/plasmalogen-deficient human fibroblasts [178]. These findings resulted in the proposal that plasmalogens can act as scavengers of ROS, serving in a protective role.

Although compelling, the lack of peroxisomes and peroxisomal functions in the mutant cells clouded the interpretation of the initial studies. However, the development of a plasmalogen-deficient CHO mutant, with normal peroxisomal function, helped to clarify the issue [42]. This mutant, NRel-4, accumulate identical or slightly less P12, than wild-type cells, yet still display hypersensitivity to P12/UV exposure. Also, restoration of plasmalogens with HG supplementation completely restored resistance to wild type-like values.

Additional evidence for plasmalogens' role as scavengers of ROS come from another series of plasmalogen-deficient variants isolated from a murine macrophage-like cell line, RAW 264.7. Both variant strains, RAW.12 and RAW.108, were exposed to electron transport inhibitors, such as antimycin-A and cyanide, in glucose-deficient buffers (chemical hypoxia). This results in the same sequence of events that occur in cells exposed to anoxia/reperfusion, including ROS generation [179,180] and cell death. Both RAW.12 and RAW.108 cells were damaged much more rapidly during exposure to chemical hypoxia [79] as well as other ROS generators. These cells could be rescued by an α-tocopherol analogue, Trolox C, or plasmalogen restoration.

This latter study was important in that it identified the vinyl ether double bond as the crucial element in protection. AG supplementation (1-hexadecylglycerol in this case) recovered plasmenylethanolamine levels to only RAW.108 and rescued only RAW.108. Due to the loss of the Δ1′desaturase in RAW.12, HG resulted only in an accumulation of plasmanylethanolamine and it did not rescue these cells. Restoration of plasmenylethanolamine levels to RAW.12 by supplementation of the growth medium with 1-alk-1-enyl-*sn*-glycerol (1-AEG) restored plasmenylethanolamine levels and resulted in wild type-like resistance to chemical hypoxia and other ROS generators.

7.5.2. In vitro model systems

A number of studies have demonstrated the ability of plasmalogens to protect lipoproteins and model membranes from radical-mediated damage. Engelmann et al. [181] determined that the plasmalogen content of low-density lipoprotein (LDL) was similar to α-tocopherol (10^{-8} moles/mg LDL protein) and the rate of loss of both during exposure to the peroxyl radical-generating agent, 2,2′-azobis-(2-amidinopropane hydrochloride; AAPH) or copper were similar. Also, the addition of lysoplasmenylethanolamine or lysoplasmenylcholine to the LDL particles lengthened the lag phase for the copper-induced oxidation of conjugated dienes (a measure of oxidation of LDL particles via lipid peroxidation). Similar findings were reported by Jurgens et al. [182].

Reiss et al. [183], using a model membrane system composed of detergent micelles containing ester and plasmalogen phospholipids of known hydrocarbon composition, monitored the oxidative degradation of phospholipids using ^{1}H-NMR. In this system, plasmalogens markedly delayed the oxidative degradation of intrachain double bonds, probably due to the interaction of vinyl ether double bonds with initiating peroxyl radicals as well as with products generated by initial oxidation of polyunsaturated fatty acids. Furthermore, the products of vinyl ether oxidation did not propagate the peroxidation of polyunsaturated fatty acids; plasmalogens functioned as "chain breakers". Hahnel et al. [184] used both ^{1}H-NMR and chemical analysis to show that plasmalogen enrichment of lipoprotein particles and micelles protected peroxyl radical-mediated oxidation of polyunsaturated fatty acids. While plasmenylethanolamine was as effective as α-tocopherol in LDL particles, it was less effective in the micellar system. Still, based upon the relative

abundance of the two, they proposed that plasmalogens are at least as important as α-tocopherol in scavenging peroxyl radicals within the cell.

8. Plasmalogens in disease

Other than the human plasmalogen-deficiency disorders, plasmalogens have been implicated in a variety of disease states, including aging, Alzheimer's disease, and heart disease and/or myocardial infarct. Evidence of changes in plasmalogen status or enhanced breakdown of plasmalogens due to these conditions has been given. However, the significance of these changes is not clear. Are plasma-logen loss and/or the generation of plasmalogen-specific metabolites contributing factors toward the pathology associated with these conditions or are they merely results of the disease progression?

8.1. Neuronal degeneration and dysfunction

A number of diseases, including aging, are characterized by degeneration of neuronal function as a result of tissue damage. In some, changes in plasmalogen status or accumulation of plasmalogen breakdown products have been documented. Below, are examples of changes in plasmalogen status being linked to disorders associated with neurological dysfunction or degeneration.

8.1.1. Inborn errors of plasmalogen biosynthesis

The most prevalent reports linking plasmalogens and neurological dysfunction are the descriptions of patients suffering from the human inborn plasmalogen deficiencies [114–116]. Invariably, these patients display some level of mental retardation. The nature of the dysfunction is still unclear, but there does appear to be a direct link between plasmalogen or ether lipid loss and neurological dysfunction in these cases. In some instances, demyelination is also associated, although this is not invariant [118].

8.1.2. Niemann-Pick disease

A recent report of reduced plasmalogen levels in the brain of patients with Niemann-Pick Type C disorder, an inborn error in cellular trafficking of cholesterol [185]. Although plasmalogen loss may not be the primary lesion, it may be a contributing factor to the progressive neurological dysfunction associated with this disease.

8.1.3. Alzheimer's disease

In 1995, Ginsberg et al. [186] reported the specific loss of plasmenylethanolamine in post-mortem brain from patients who had suffered from Alzheimer's disease. This decrease was more prevalent in the affected regions of the brain. The reason for this decrease may be due to activation of plasmalogen-selective phospholipase A_2 in the affected regions as proposed by Farooqui et al. [187], the resulting metabolites such as arachidonate and lipid peroxides contributing to cellular damage. Alternatively, changes in plasmalogen levels may alter membrane stability, contributing to membrane compromise and cell death [188]. Finally, the fact that there is increased oxidation of proteins, lipids and DNA in Alzheimer's disease is evidence that oxidative damage is a major

factor in the pathology [189]. It is possible that the loss of plasmalogen in Alzheimer's brain tissue is a result of oxidation, given the sensitivity of the vinyl ether to reactive oxygen species.

8.1.4. Down syndrome

Murphy et al. [190] recently examined the relative phospholipid composition and levels in post-mortem frontal cortical and cerebellar gray matter from older Down syndrome patients (age range 38–68 years). They reported a 35% decrease in plasmenylethanolamine as well as a 37% decrease in phosphatidylinositol in these sections in the Down syndrome compared to normal controls. Total phospholipid content and cholesterol content were also reduced by 20%. A number of biological markers for oxidative stress are present in Down syndrome patients including elevated 8-hydroxy-2′-deoxyguanosine (damage to DNA), and malondialdehyde, a product of lipid peroxidation [191]. Also, microglia isolated from trisomy 16 mice (a murine Down syndrome model) produced much larger amounts of superoxide than microglia from normal mice when stimulated with either phorbol ester or opsonized zymosan [192]. These findings suggest that heightened oxidative stress may be associated with Down syndrome. The loss of plasmalogens in these patients may be another marker indicating oxidative stress.

8.2. Ischemia-reperfusion injury

During ischemia-reperfusion, a number of events occur which may result in cellular damage and death. These include the activation of phospholipase A_2s, production of reactive oxygen species, and increased flux of calcium across the plasma membrane into the cell. With reports that some of these phospholipases are plasmalogen-selective, the identification of plasmalogens as possible antioxidants, and the identification of plasmalogen-derived metabolites with biological activity, one can speculate that plasmalogens are significant factors in cell damage during events such as stroke or myocardial infarct. The question becomes, do plasmalogens protect cells under this stress, or do they contribute to cell damage?

The action of phospholipase A_2, activated during hypoxia, results in lysoplasmenylcholine and/or plasmenylethanolamine. Lysoplasmenylcholine accumulation has been documented in hypoxic cells [146,193] This phospholipid inhibited Na^+/K^+ ATPase in renal proximal tubules [146] and caused action potential abnormalities in ventricular myocytes [193,194]. Additionally, radical-mediated plasmalogen breakdown products, including α-hydroxyaldehydes [195], aldehydes, lysophospholipids and formic acid [177], could be considered toxic byproducts of the reactive oxygen generated during hypoxia and hypoxia/reperfusion. These findings suggest that plasmalogens play a significant role in the pathology associated with hypoxia and or hypoxia/reperfusion.

Alternatively, plasmalogens could be looked at as beneficial under these conditions. If plasmalogens do function as antioxidants, their presence may ameliorate the damage caused by the reactive oxygen species component. As mentioned previously, plasmalogen-deficient cell lines were more sensitive to conditions that result in reactive oxygen-mediated cell damage including chemical hypoxia. Additionally, elevating plasmalogen levels in human endothelial cells resulted in dramatically increased survival during hypoxia [196]. Although it is not certain that plasmalogens were rescuing these cells through their antioxidant capabilities, it supports the notion the plasmalogens can serve in a beneficial function under hypoxic stress.

9. Conclusions

Significant progress has been made over the past 15 years in the area of plasmalogens, however, there is still a great deal we do not know. There has been some success in isolating proteins and genes that are important for the biosynthesis of plasmenylethanolamine. Still, we do not know what controls the levels of this phospholipid nor do we have a well-defined pathway for plasmenylcholine biosynthesis. Why has it been difficult to elevate plasmalogen levels through supplementation or over-expression of biosynthetic enzymes?

The description of human peroxisome and plasmalogen-deficiency disorders, the development of plasmalogen-deficient cell lines, and the emergence of new technologies for the chemical analysis of plasmalogen structure and metabolism have helped to identify putative roles for plasmalogens in cells or tissues, yet no function has been clearly proven. A definitive link between ether lipids and cell function can be established in some cases, but discerning which ether-lipid species is important has been difficult. We still can only speculate as to why certain tissues, such as heart, contain significant levels of plasmenylcholine while others do not. Does the choline head group impart a unique function or are they merely a way to increase plasmalogen levels that are tolerated by the cell? Certainly, the vinyl ether, which defines plasmalogens, must be crucial to their role(s). The development of additional plasmalogen-deficient cell lines and plasmalogen-deficient animal models will allow the testing of the putative functions attributed to these lipids.

Ultimately, we must determine if plasmalogens are beneficial during certain disease processes or whether they, in fact, exacerbate these situations. If they are beneficial, we must determine if, and by what means, these compounds can be increased within tissues and we can then determine if this is beneficial under pathological conditions.

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