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Ether Phospholipid Biosynthesis: An Overview

Abstract

Ether lipid deficiency in human inborn errors of metabolism characterized by a general impairment of peroxisome assembly provided definitive proof for the indispensable role of this subcellular organelle in the process of ether lipid synthesis. The enzymology of ether lipid synthesis and its topology in peroxisomes is briefly discussed. This includes the complete purification and identification of the peroxisomal enzyme responsible for glycero-ether bond formation as well as the selectivities of enzymes involved in the formation of platelet-activating factor, a bioactive glycerophospholipid of which the biological activity is greatly dependent on the presence of the glycero-ether linkage.

Introduction

Mammalian ether phospholipids can be distinguished in two groups, i.e. those with a saturated ether linkage at the *sn*-1-position of the glycerol backbone and those with an α,β-unsaturated ether linkage at that position. More specifically these compounds are denoted as 1-alkyl-2-acyl- and 1-alkenyl-2-acyl glycerophospholipids, respectively. The alcoholic moiety attached to the phosphate group is almost exclusively confined to ethanolamine or choline. By analogy to the official names phosphatidylethanolamine and phosphatidylcholine for 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine and 1,2-diacyl-*sn*-glycero-3-phosphocholine the respective ether linked species are denoted by plasmanylethanolamine/choline for the compounds carrying an *sn*-1-alkyl group and by plasmenylethanolamine/choline for the structures with an *sn*-1-alkenyl group. This official nomenclature will be used throughout this review.

The biosynthesis of ether lipids was elucidated in the early seventies mainly through the work of Hajra, Snyder and Friedberg and their co-workers (see [11, 33] for reviews). The process starts with the acylation of dihydroxyacetone phosphate (DHAP) by a specific acyltransferase. The glycero-ether bond is then formed by the enzyme alkyl-DHAP synthase in a reaction in which the acyl group in acyl-DHAP is replaced by a long-chain alcohol. In this unique reaction

both oxygens in the ester bond leave with the alkyl chain and the oxygen for the ether bond to be formed is donated by the long-chain alcohol [5, 7]. Next the keto group in alkyl-DHAP is reduced to a hydroxyl function which then becomes acylated. 1-Alkyl-2-acyl phospholipids are then formed by the same enzymes that catalyze the successive steps, i.e. dephosphorylation and introduction of a phosphoethanolamine or phosphocholine group, in the biosynthesis of diacylphospholipids. The final step then is a desaturase reaction to convert plasmanylethanolamine into plasmenylethanolamine. This reaction appears to be confined to the ethanolamine compounds. For the synthesis of plasmenylcholine an additional reaction sequence involving the removal of the phosphoethanolamine group from plasmenylethanolamine and the introduction of a phosphocholine group into the resulting plasmalogenic diglyceride analogue appears to be required [21].

The subcellular localization of the first reactions in ether lipid synthesis as catalyzed by DHAP-acyltransferase (DHAP-AT) and alkyl-DHAP synthase initially caused considerable confusion. This was caused by the use of different biological materials for these studies. In liver the enzymes were recovered in the crude mitochondrial fraction whereas in Ehrlich ascites cells a microsomal localization was deduced. This discrepancy was resolved when Hajra and co-workers [10] discovered that neither was correct and that the enzymes were in fact highly enriched in peroxisomes. The indispensable role of peroxisomes in ether lipid synthesis was fully confirmed by the discovery of ether lipid deficiency in Zellweger patients [18]. Before discussing these studies and the analyses of phospholipid subclass composition as well as the enzymology of ether lipid synthesis in more detail we shall first deal with some of the physico-chemical properties of ether phospholipids.

Results

Physical-chemical properties of phospholipid subclasses.

Plasmalogens occur widespread in nature, especially in the mammalian kingdom. For still unknown reasons plasmenylcholines are less abundant than plasmenylethanolamines. Despite this widespread occurrence relatively little is known about the specific functions of ether phospholipids in general and plasmalogens in particular. The only notable exception is the structural membrane phospholipid plasmanylcholine. This 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine serves as a precursor for platelet-activating factor [13] after the long-chain acyl group at the *sn*-2-position has been replaced by an acetyl group through successive action of a phospholipase A_2 and an acetyltransferase. Platelet-activating factor is a functionally important lipid mediator with many and diverse biological effects and its function is critically dependent on the presence of the ether linkage at the *sn*-1-position [26].

The overall physical-chemical properties of ether phospholipids are similar to

those of the corresponding diacyl phospholipids. Small differences exist, however, in the packing [32] and phase transition [17,22] properties of the various subclasses (Table I). These differences in phase transition temperatures are mainly observed for the ethanolamine subclasses in the conversion of lamellar to Hexagonal II phases, where the transition temperature for the plasmenyl subclass is 2-fold lower than that for the diacyl subclass. Such differences are hardly observed for the gel to liquid crystalline phase conversion. The packing characteristics of the ethanolamine subclasses, as indicated by the cross-sectional area the molecules occupy in monolayers at 30 dynes/cm, are mainly determined by polar headgroup interactions and consequently do not differ for the various subclasses. Differences in this parameter are observed for the choline phospholipid subclasses, with the plasmenyl species exhibiting a considerably smaller cross-sectional area than the diacyl species. These differences can be explained by the structures of these species as determined by X-ray and NMR for phosphatidylcholine and by NMR techniques for plasmenylcholine [12]. The characteristic differences are a different orientation of the glycerol backbone, which is perpendicular to the plane of the membrane in case of the diacyl species and bent in the case of the plasmenyl species. Secondly, due to a characteristic bent in the proximal end of the *sn*-2-acyl chain in diacyl species there is a large spacial difference in the location of the carbonyl carbons of the *sn*-2- and *sn*-1-fatty acids. In plasmenylcholine the aliphatic chains are in much closer spacial proximity with the carbon numbers of the aliphatic chains linked up and a nearly equidistant 3 Å proximity of the β-vinyl ether proton with the α- and β-methylene protons of the *sn*-2-aliphatic chain [12]. These differences explain the reduced cross-sectional area observed for plasmenylcholine in comparison to phosphatidylcholine. On the other hand, these differences in physical-chemical behaviour of the respective phospholipid subclasses have provided no clues for their specific functions.

Other approaches using plasmalogen-deficient Chinese hamster ovary cell lines have suggested a function for plasmalogens as membrane-associated protectants against certain oxidative stresses [39]. This matter will be discussed by Zoeller elsewhere in this volume.

Table I. Structural properties of phospholipid subclasses

Abbreviations: -GPC, *sn*-glycero-3-phosphocholine; -GPE, *sn*-glycero-3-phosphoethanolamine; L, liquid crystalline lamellar phase; G, gel lamellar phase; H_{II}, hexagonal phase.

Property		Diacyl-	Alkylacyl-	Alkenylacyl-
Cross-sectional area (30 dynes/cm); $Å^2$	-GPC	67.7	61.2	59.9
	-GPE	55.0	55.4	55.0
Phase transitions				
$L \rightarrow H_{II}$, °C	-GPE	64	54	33
$G \rightarrow L$, °C	-GPE	30	29.5	26

A combination of literature findings indicating that morphologically detectable peroxisomes were absent in liver and kidney samples of Zellweger patients [9] and the results from cell fractionation experiments suggesting the peroxisomal localization of ether lipid biosynthetic enzymes [10] predicted that ether lipids might be deficient in Zellweger patients. This was experimentally confirmed by analysis of the plasmalogen content of control and ZS tissues such as brain, kidney, liver, muscle and heart [18]. The procedure involved 2-dimensional thin-layer chromatography. After chromatography in the first dimension the thin-layer plate was subjected to treatment with HCl-fumes to cleave the vinyl ether linkage in plasmalogens. Subsequent chromatography in the second direction then separated each phospholipid class into a lysophospholipid and long-chain aldehyde component, both equivalent to the plasmenyl phospholipid class, and an acid-stable mixture of the diacyl- and alkyl-acyl subclasses of that phospholipid class. The results showed the virtual absence of the lysophospholipid and long-chain aldehyde spots derived from plasmalogen species in the tissues of Zellweger patients. Further analysis of the acid-stable mixtures obtained in this procedure by alkaline hydrolysis indicated that it was almost exclusively composed of diacylphospholipids. This showed, as expected,

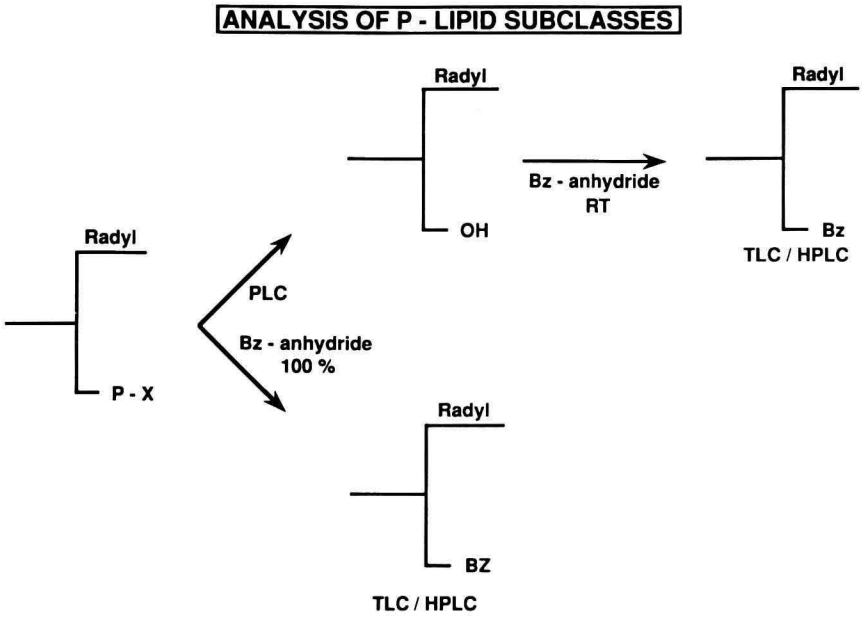

Fig. 1. Schematic representation of phospholipid subclass analyses. Upper part: two-step procedure involving phospholipase C (PLC) degradation, isolation of radyl-acyl glycerols and subsequent chemical benzoylation. Lower part: chemical procedure involving dephosphorylation and benzoylation without isolation of intermediates.

that not only plasmalogens were absent but that a general deficiency in ether phospholipids was manifested in Zellweger syndrome [18].

Analysis of phospholipid subclasses

The above described procedure for the complete analysis of phospholipid subclass composition involving the determination of the plasmenyl species after acid treatment and subsequent application of alkaline hydrolysis to differentiate between phosphatidyl- and plasmanyl species is rather laborious. Direct separation of the intact phospholipids in their three subclasses cannot be achieved due to the fact that the chromatographic behaviour is mainly determined by the polar headgroups. Separation into the three subclasses can only be achieved

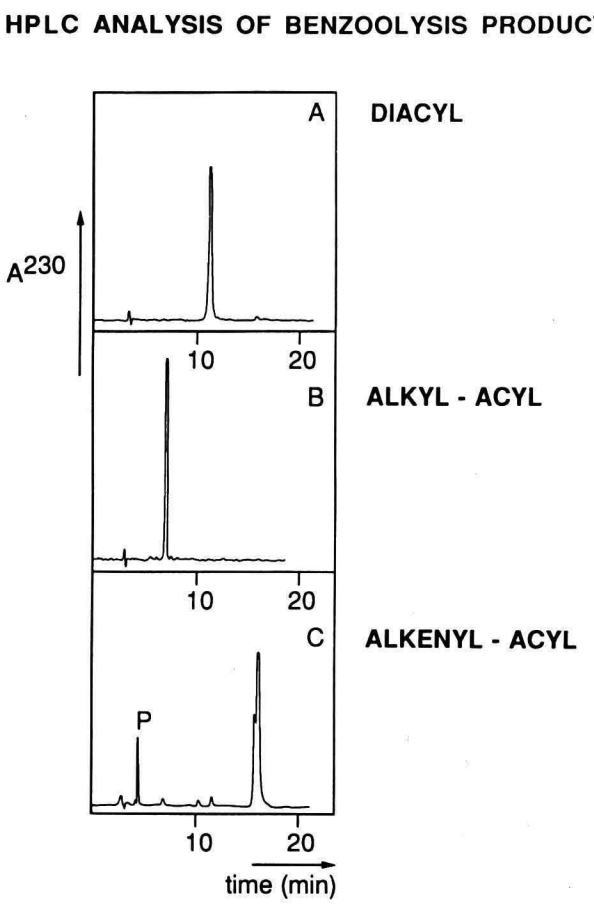

Fig. 2. High performance liquid chromatography of benzoolysis products obtained from model phospholipid subclasses. A: diacylglycerobenzoate derived from phosphatidylcholine
B: alkyl-acylglycerobenzoate derived from plasmanylcholine
C: acylglycerodibenzoate derived from plasmenylcholine with P is the addition product of benzoic anhydride and the long chain aldehyde derived from plasmenylcholine.

after removal of the polar headgroup. As indicated in figure 1 two methods are now available. The first method involves removal of the polar headgroup by phospholipase C and subsequent benzoylation to yield a mixture of 1-radyl-2-acyl-3-benzoyl glycerols. By TLC or HPLC procedures these can be separated into the individual components which can be quantitated by UV-absorption due to the presence of the benzoyl group [1]. This 2-step procedure requires control TLC analyses to verify complete conversion by phospholipase C and isolation of the resulting 1-radyl-2-acyl glycerol mixture prior to introduction of the benzoyl group. Since it was known that phospholipids can be chemically dephosphorylated by acetolysis in a mixture of acetic anhydride and acetic acid to yield the radyl-acyl-acetyl glycerols [27] we wondered whether treatment with benzoic anhydride and benzoic acid would similarly yield the radyl-acyl-benzoyl glycerols. In fact it turned out that with some modifications, which will be described in detail elsewhere, a benzoolysis process could be developed combining the dephosphorylation and benzoylation step in a single tube. Since the vinyl ether linkage of plasmalogens is labile under these conditions the procedure applied to individual phosphatidyl-, plasmanyl-, and plasmenylcholines yields diacylglycerobenzoates, alkyl-acylglycerobenzoates and acylglycero-dibenzoates, respectively. As shown in figure 2, these can be separated and quantitated by high performance liquid chromatography.

Postnatal and prenatal diagnoses of peroxisomal disorders based on deficient ether lipid biosynthesis

The discovery of ether lipid deficiency in Zellweger syndrome initiated studies to develop diagnostic procedures based on this deficiency. Eventually this was done by measuring plasmalogen biosynthesis from radioactive hexadecanol in fibroblasts and amniotic fluid cells. Hexadecanol can be directly incorporated into ether lipids by replacing the acyl group in acyl-DHAP. It can also be oxidized to palmitic acid and become as such incorporated into phospholipids. In control fibroblasts about 45% and 5% of the total radioactivity incorporated in phospholipids was recovered in the alkenyl chains of plasmenylethanolamine and plasmenylcholine, respectively. Thus, fibroblasts synthesize their own plasmalogens. Independent experiments using delipidated media showed that plasmalogens were not taken up from the culture medium [30]. This process of *de novo* ether lipid biosynthesis was impaired in Zellweger fibroblasts where the percentages incorporation into the respective alkenylchains were about 6-fold reduced [28]. Similar observations were made in amniotic fluid cells, except that in these cells from controls somewhat more radioactivity was incorporated into the alkenyl chain of plasmenylcholine than in fibroblasts (figure 3). Therefore, such hexadecanol incorporation studies differentiated between normal and Zellweger syndrome and could be used for postnatal and prenatal diagnosis of this syndrome.

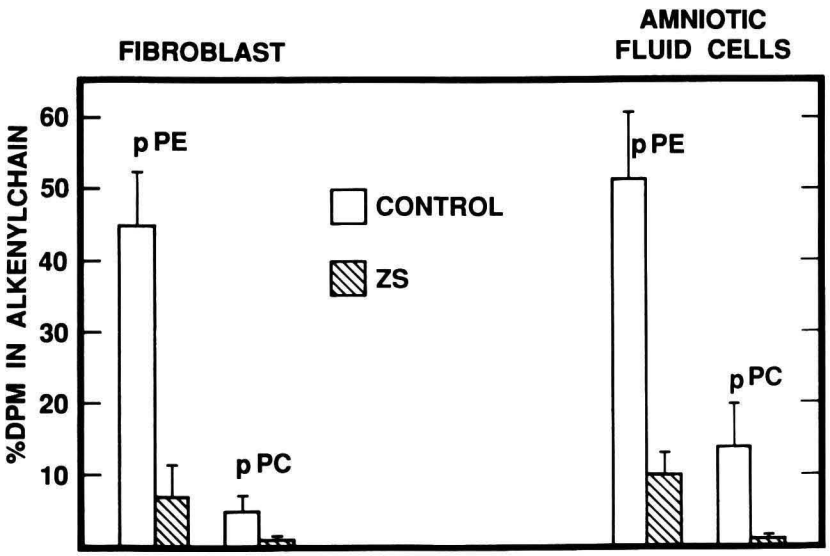

Fig. 3. Hexadecanol incorporation into alkenyl chains of plasmenylethanolamine and plasmenylcholine of control and Zellweger syndrome fibroblasts and amniotic fluid cells.

The impaired biosynthesis of plasmalogens from hexadecanol and the general deficiency of ether phospholipids as deduced from phospholipid subclass analysis in Zellweger syndrome provided definitive proof that in humans peroxisomes are indispensable for ether lipid biosynthesis. Subsequent experiments using cell-free systems indicated that both DHAP-AT [31] and alkyl-DHAP-synthase [29] were deficient in Zellweger syndrome, underscoring the peroxisomal localization of both enzymes.

Topology of ether lipid biosynthesis in peroxisomes

The peroxisomal contribution to ether glycerolipid biosynthesis is schematically represented in figure 4 (see [3] for references). Fatty acid activation to acyl-CoA esters takes place by a peroxisomal acyl-CoA ligase, the active site of which faces the cytosolic compartment. By contrast, DHAP-AT and alkyl-DHAP synthase are resistant to proteolysis unless detergent is used to destroy the membrane barrier [14,19]. This suggests that the active sites of these enzymes are located at the inner aspect of the peroxisomal membrane. This conclusion, as least for DHAP-AT, is supported by experiments showing the latency of this enzyme in selectively permeabilized fibroblasts but not in sonicated cells [38]. Alkyl-DHAP then traverses the peroxisomal membrane where it becomes reduced to alkyl-glycero-3-phosphate (alkyl-G3P) by an acyl/alkyl-DHAP reductase at the cytoplasmic leaflet of the membrane. The acylation of alkyl-G3P at the *sn*-2-position and further steps in ether lipid biosynthesis are

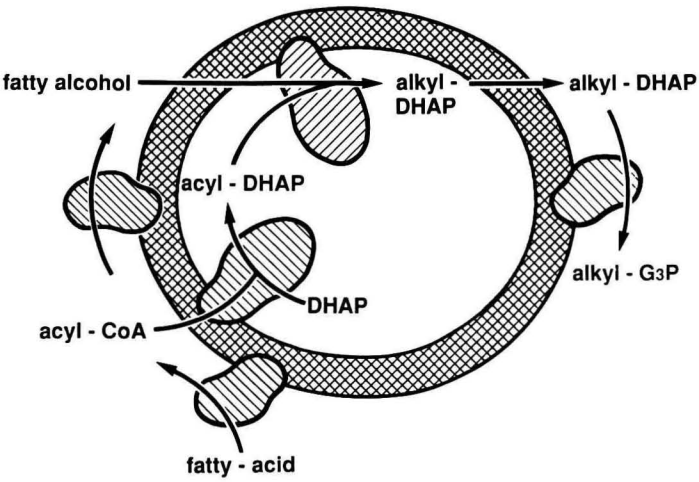

Fig. 4. Topology of peroxisomal enzymes involved in ether glycerolipid biosynthesis. Reproduced with permission from [3].

catalyzed by enzymes that are not located in peroxisomes but in the endoplasmic reticulum.

The sidedness of DHAP-AT and alkyl-DHAP synthase as indicated in figure 4 is solely based on proteolysis experiments. It, therefore, is most likely correct for the active sites of the enzymes but the proteolysis experiments do not rule out the possibility that the enzymes may be integral membrane proteins that span the membrane and expose part of their structure at the cytoplasmic face of the membrane. This putative part then may either not be susceptible to the proteolytic enzymes that have been used or may be removable without affecting enzymatic activity of the peroxisomal matrix domains. In fact, several observations support an integral membrane character of both DHAP-AT and alkyl-DHAP synthase. To distinguish between integral and peripheral membrane-associated proteins a one-step carbonate extraction procedure has been developed [8]. This procedure effectively releases soluble and peripheral membrane proteins from organelles and yields a preparation consisting of membrane lipids and integral membrane proteins. This convenient procedure is often too harsh if one has to rely on enzymatic activity to determine the distribution of a protein over the fractions (compare table II). In such cases the distribution can often be established by an alternative technique involving Triton X-114 phase separation [16]. Briefly, an organelle sample in detergent solution is layered on top of a sucrose solution and phase separation is induced by increasing the temperature to 30° C. Centrifugation then yields a buffer phase containing soluble

Table II. Triton X-144 phase separation of peroxisomal enzymes

For experimental details see [16].

Enzyme	Total recovery		%Distribution	
	Carbonate treatment	Phase separation	Water phase	Detergent phase
Catalase	0	91	95	5
Thiolase	4	116	79	21
Acyl-CoA ligase	0	82	9	91
DHAP-AT	0	0	-	-
Alkyl-DHAP synthase	0	45	5	95

and peripheral membrane proteins and a small detergent phase containing integral membrane proteins. When applied to peroxisomes (Table II) the matrix enzymes catalase and thiolase were mainly recovered in good yield in the water phase. By contrast, acyl-CoA ligase and alkyl-DHAP synthase were almost exclusively recovered in the detergent phase, suggesting an integral membrane character for these enzymes. Due to loss of activity in even this mild procedure no information could be obtained for DHAP-AT. However, the integral membrane character of this protein is supported by the requirement of detergents to effectively solubilize it from peroxisomal membranes [37].

Solubilization experiments also provided an additional argument for the integral membrane character of alkyl-DHAP synthase. As shown in Fig. 5, treatment of an organelle fraction from guinea pig liver enriched in peroxisomes (L-fraction) with 1.0 M KCl failed to solubilize the enzyme. Detergent alone

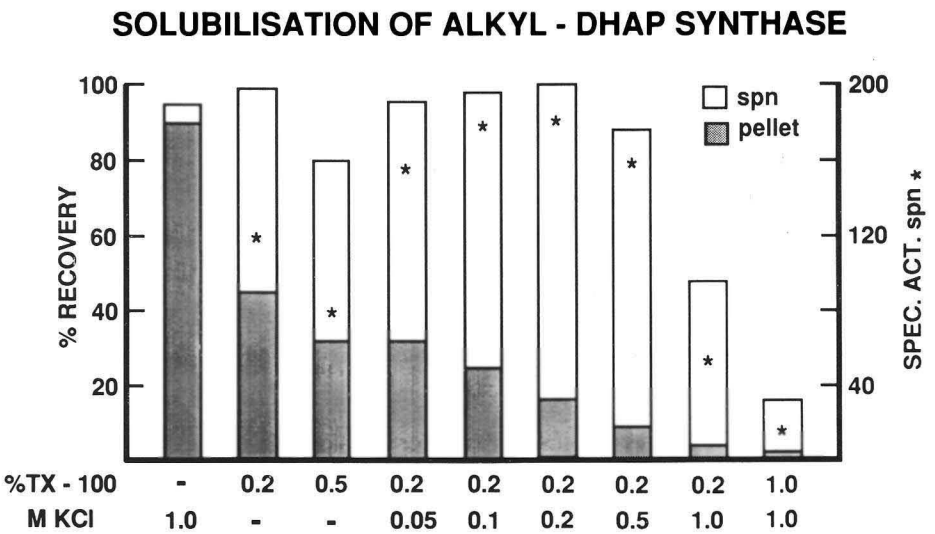

Fig. 5. Solubilization and recovery of alkyl-DHAP synthase from guinea pig liver at various detergent and salt concentrations. Specific activities are expressed in pmol · min^{-1} · mg^{-1}.

solubilized only about half of the total activity. Good solubilization, as indicated by the open bars, with nearly 100% recovery of total enzyme activity was only obtained in the presence of 0.2% Triton X-100 and from 0.2 to 0.5 M KCl. As indicated by the symbols in the open bars, these mixtures also gave the highest specific activities of the enzyme in the solubilized fraction. Higher salt and detergent concentrations resulted in virtually complete solubilization but at the same time in inactivation of the enzyme as evidenced by considerable decreases in both total activity recovered and the specific activity of the solubilized enzyme.

Purification of alkyl-DHAP synthase

The purification of several peroxisomal proteins, notably those of peroxisomal β-oxidation but also including some peroxisomal membrane proteins, was greatly facilitated as a result of induction of the organelle and those proteins by peroxisome proliferators such as clofibrate and plasticizers. This prompted us to investigate whether also the enzymes from ether lipid metabolism could be induced [15]. Fig. 6 conforms the well-known increase in the specific activity of the peroxisomal β-oxidation process in rat liver homogenates after clofibrate and plasticizer feeding. These increases persist in purified peroxisomes indicating a selective induction of these enzymes over total peroxisomal proteins. As in the case of catalase, an approximately 2-fold increase in the specific activity of DHAP-AT is observed under these conditions in homogenates. Presumably this is caused by an increase in the number of peroxisomes as no increase in the specific activities of these enzymes is observed in purified peroxisomes. Alkyl-DHAP synthase did not increase at all. The strategy of enzyme induction to facilitate purification can therefore not be applied to the enzymes of ether lipid biosynthesis, certainly not for alkyl-DHAP synthase, the most characteristic enzyme from the pathway that introduces the glycero-ether linkage.

We recently succeeded in the complete purification of alkyl-DHAP synthase from guinea pig liver [40]. The purification involved solubilization of the enzyme by the procedures outlined in figure 5 followed by QAE-Sephadex A-25-, Matrex Gel Red A-, hydroxyapatite- and/or phosphocellulose chromatography. As can be seen in Fig. 7, the preparation obtained after these steps, designated by SM, still contained multiple protein bands. These could best be resolved by Concanavalin A chromatography. Most of the proteins in the starting material SM ran through the column as shown in the SDS-PAGE of fractions 3 and 5 eluted from the column. Enzyme activity was slightly retarded and eluted in fractions 5 to 17 with highest activities in fractions 7 to 10. The only protein band in the SDS-PAGE of the eluted fractions that correlates in its intensities with enzymatic activity is a 65 kDa band, suggesting this protein represents alkyl-DHAP synthase. The main contaminating proteins with lower molecular weights, notably a 60 kDa band, appear to be somewhat less retarded by the column. Advantage was taken from this observation by applying the starting material in subsequent purifications in a buffer with a lower percentage Triton

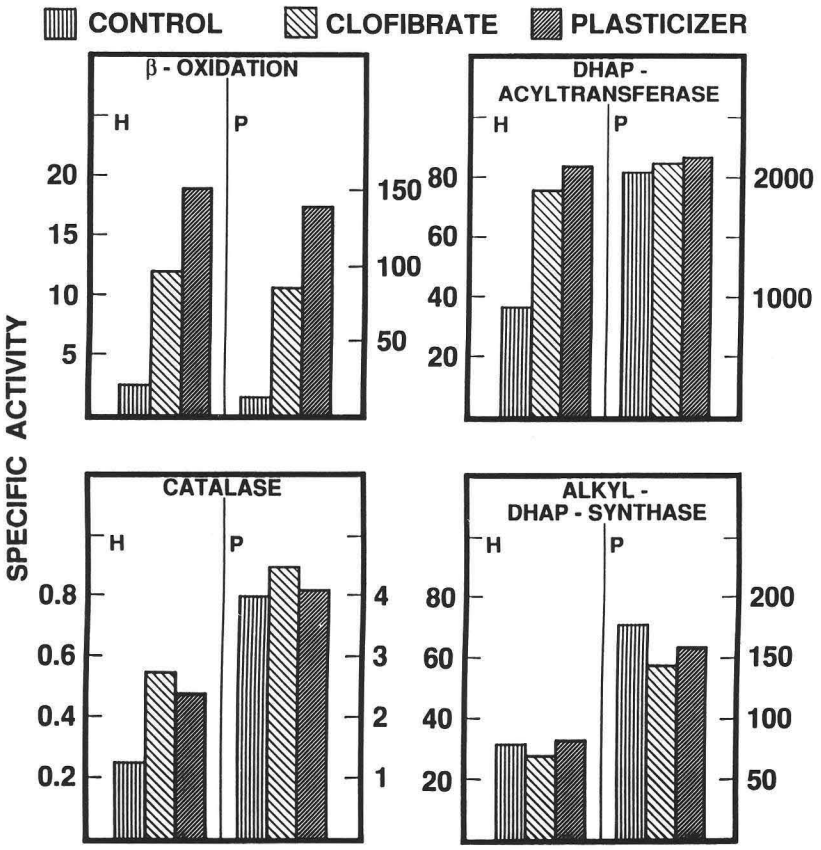

Fig. 6. Effect of peroxisome proliferation on specific activities of the indicated peroxisomal enzymes. Enzyme activities were determined in liver homogenates (H) and purified peroxisomes (P) from control, clofibrate- of plasticizer-treated rats. Specific activities are expressed in nmol · min^{-1} · mg^{-1} (β-oxidation), micromol · min^{-1} · mg^{-1} (catalase) or pmol · min^{-1} · mg^{-1} (DHAP-AT and alkyl-DHAP synthase). Reproduced with permission from [4].

X-100. Under these conditions (Fig. 8) all of the contaminating proteins in SM could still be eluted by this buffer as evidenced by their presence in fraction B2. The 60 kDa contaminating band was more retarded and eluted in buffer fractions up to B8. Enzyme activity remained bound to the column and was subsequently eluted in a broad peak after application of a Triton X-100 gradient in buffer. Fractions with enzymatic activity showed only one protein band in intensities correlating with enzymatic activity. We conclude from these experiments that alkyl-DHAP synthase activity resides in a single polypeptide chain with a molecular mass of 65 kDa. The above ConA chromatography experiments should not be interpreted to indicate that alkyl-DHAP synthase is a

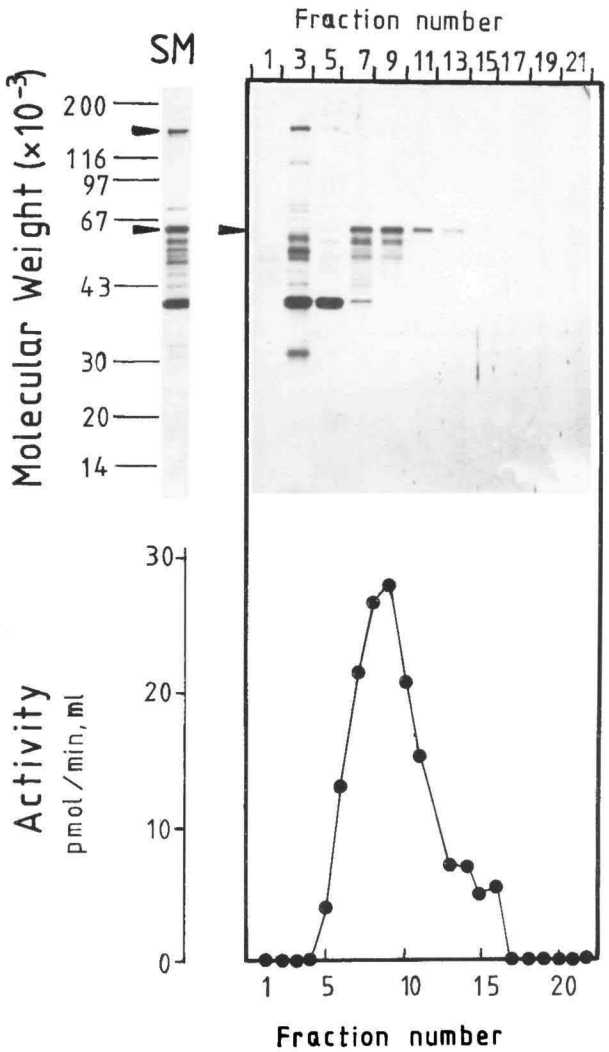

Fig. 7. Concanavalin A chromatography of alkyl-DHAP synthase.The starting material SM obtained after several chromatographic procedures was chromatographed over a Concanavalin A HPLAC column. Lower part: Elution of enzymatic activity. Upper part: SDS-PAGE of starting material SM and selected eluted fractions as indicated. Alkyl-DHAP synthase is indicated by an arrow head left from the main SDS gel.

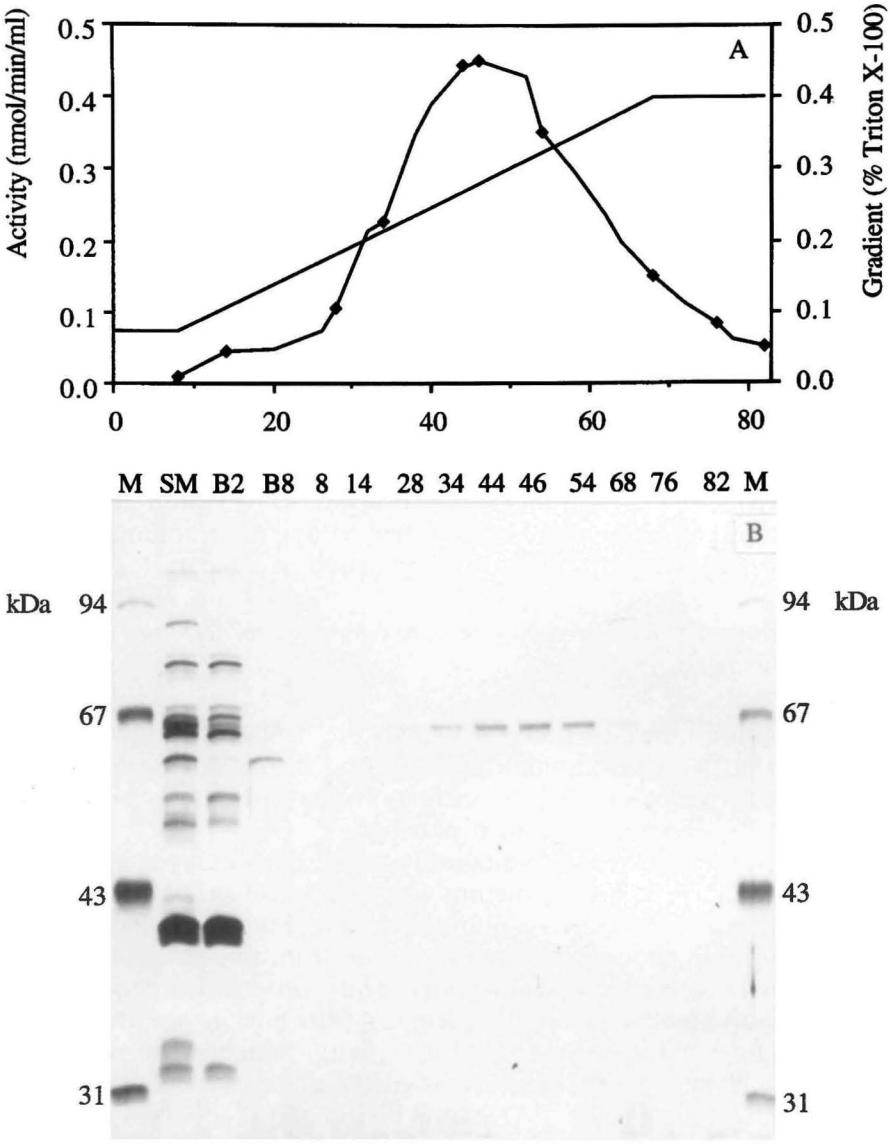

Fig. 8. Purification of alkyl-DHAP synthase by ConA chromatography. The starting material SM was applied to the column in a buffer containing 0.1% Triton X-100. Contaminating proteins were eluted in this buffer in fractions B1 through B8. Enzyme was then eluted by application of a Triton X-100 gradient ranging from 0.1% to 0.4% in buffer. Upper part A: Elution pattern of enzymatic activity. Lower part B: SDS-PAGE of marker proteins M, starting material SM and selected column fractions as indicated. Reproduced with permission from [40].

Table III. Properties of peroxisomal enzymes involved in ether lipid biosynthesis

Abbreviations: gp, guinea pig; h, human; I.e.p., isoelectric point

Enzyme	EC-number	Source	Mol. weight	I.e.p.	Ref.
AcylCoA:DHAP acyltransferase	2.3.1.42	gp liver	69 kDa	5.1-5.4	37
		h placenta	65 kDa	5.1-5.3	25
Alkyl-DHAP synthase	2.5.1.26	gp liver	65 kDa	5.8-6.0	40
NADPH: acyl/alkyl-DHAP oxidoreductase	1.1.1.100	gp liver	60 kDa	-	6

glycoprotein as we have evidence [40] that the binding is based on interactions with the core material of the column.

All three peroxisomal enzymes involved in the initial steps of ether lipid synthesis, i.e. DHAP-AT [25,37], alkyl-DHAP synthase [40] and acyl/alkyl-DHAP reductase [6] have now been purified to apparent homogeneity. Some of the properties of these enzymes are summarized in table III.

Platelet-activating factor: Deficiency in peroxisomal disorders and enzyme selectivities in its synthesis

After it had been established that ether lipids are deficient in disorders with a general impairment in peroxisomal functions and in the rhizomelic form of chondrodysplasia punctata [36], it became of interest to study platelet-activating factor (PAF) biosynthesis in such patients.

Platelet-activating factor is a biologically active phospholipid with many and diverse effects [13, 26, 35]. Its structure was elucidated over a decade ago as being 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. Studies on the structure-function relationship showed that any deviation from this structure resulted in a compound with lower biological activity. Thus, progressive demethylation of the choline group and increases in the length of the acyl group at the *sn*-2-position led to analogs with progressively less activity. Notably, the replacement of the 1-alkyl group by a 1-acyl group reduced the platelet-activating capacity by at least 2 orders of magnitude. PAF exerts its biological activities through binding to a specific receptor that belongs to the large class of seven transmembrane domain receptors of the rhodopsin gene family. The PAF receptor has recently been cloned from guinea pig lung [24] and human leukocytes [20, 24] and consists of a single polypeptide chain of 342 amino acids.

In three different rhizomelic chondrodysplasia punctata patients PAF synthesis after A23187 Ca^{2+}-ionophore stimulation of leukocytes was reduced 600-, 7- and 17-fold, respectively, in comparison to controls. This considerable biological variation correlated nicely with the residual levels of the PAF precursor plasmanylcholine in leukocytes from these patients, which were 360-, 7- and

24-fold reduced, respectively [4]. This suggested that the level of plasmanylcholine precursor molecules in the membrane phospholipids is an important determinant for PAF synthesis. This was fully born out in subsequent studies on the selectivity of the enzymes involved in PAF synthesis upon stimulation of either leukocytes or platelets [2].

The results of these studies are summarized in Fig. 9. The ratio of 1-ether-linked and 1-ester-linked species in the choline glycerophospholipids of a given cell can be set at A. The absolute values for this ratio are cell-type specific amounting to 1.1 for leukocytes and 0.2 for platelets, respectively [2], but otherwise similar trends were observed in both cell types. These trends indicated that upon stimulation of the cells and analyses of the ether/ester ratio at the *sn*-1-position of PAF and its 1-acyl analog a 6 to 10-fold enrichment in ether-linked species was observed. This initially suggested a selectivity of the phospholipase A2 and/or acetyltransferase for ether-linked phospholipid species. However, our studies showed that this conclusion was incorrect. In fact, neither phospholipase A_2 nor acetyltransferase showed any selectivity for ether-linked species and produced lyso-PAF/lysophosphatidylcholine mixtures and PAF/acyl-analog mixtures with ether/ester ratios at the *sn*-1-position that were identical to the ratio A in the precursor molecules. The acyl analogs then become deacylated to yield a mixture 6 to 10-fold enriched in ether-linked species, giving

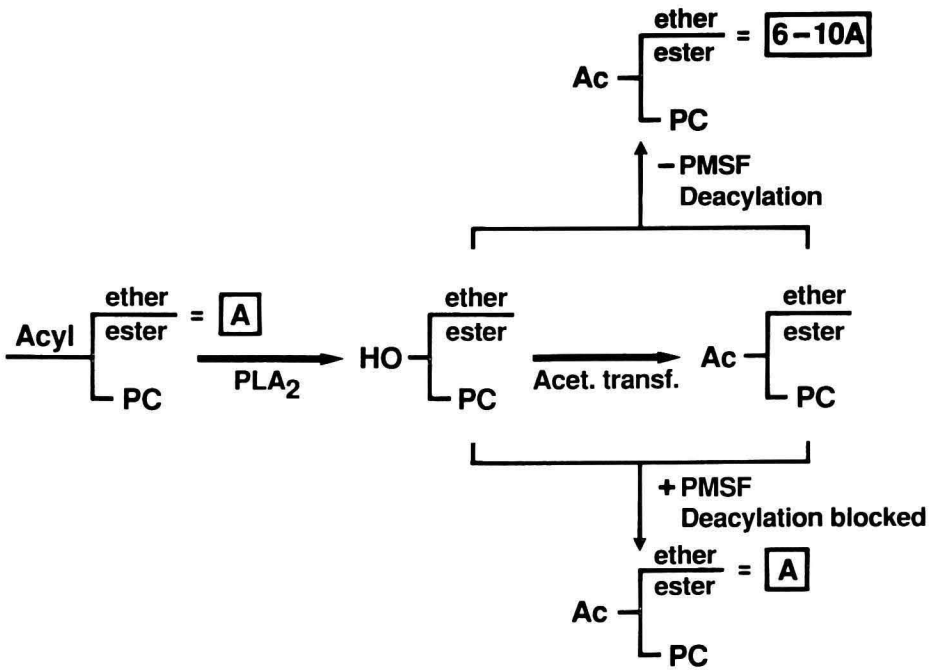

Fig. 9. Selectivities of enzymes involved in the synthesis of PAF and its acyl analog 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine. PC, phosphocholine; Ac, acetyl; PLA_2, phospholipase A_2; Acet. transf., Acetyl transferase; PMSF, phenylmethanesulfonylfluoride. Reproduced with permission from [4].

the erroneous impression of phospholipase A_2 and acetyltransferase selectivity for ether-linked species. That this is not the case followed from experiments in which the deacylation process was blocked by pretreatment of the cells with phenylmethanesulfonylfluoride. Under these conditions a mixture of PAF and its 1-acyl analog was produced with an ether/ester ratio identical to the value A of the precursor molecules in each cell type [2].

It follows from these considerations that the ratio in which PAF and its 1-acyl analog is produced upon cell stimulation is mainly governed by two parameters. The first one is the ratio in which ether-linked and ester-linked species occur in the structural membrane choline glycerophospholipids [2, 34] and the second one is the activity of the deacylase that deacylates the 1-acyl analogs [23]. The biological activity of the resulting mixture is determined by the content of the 1-ether-linked species. This stresses the importance of glycero-ether bond synthesis and hence of peroxisomes, where this process takes place, for cell physiology.

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