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Mini-review

Orphan enzymes in ether lipid metabolism

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ABSTRACT

Ether lipids are an emerging class of lipids which have so far not been investigated and understood in every detail. They have important roles as membrane components of e.g. lens, brain and testis, and as mediators such as platelet-activating factor. The metabolic enzymes for biosynthesis and degradation have been investigated to some extent. As most involved enzymes are integral membrane proteins they are tricky to handle in biochemical protocols. The sequence of some ether lipid metabolising enzymes has only recently been reported and other sequences still remain obscure. Defined enzymes without assigned sequence are known as orphan enzymes. One of these enzymes with uncharacterised sequence is plasmanylethanolamine desaturase, a key enzyme for the biosynthesis of one of the most abundant phospholipids in our body, the plasmalogens. This review aims to briefly summarise known functions of ether lipids, give an overview on their metabolism including the most prominent members, platelet-activating factor and the plasmalogens. A special focus is set on the description of orphan enzymes in ether lipid metabolism and on the successful strategies how four previous orphans have recently been assigned a sequence. Only one of these four was characterised by classical protein purification and sequencing, whereas the other three required alternative strategies such as bioinformatic candidate gene selection and recombinant expression or development of an inhibitor and multidimensional metabolic profiling.

1. Ether lipids

1.1. Chemical nature and function of ether lipids

Ether lipids (alkyl and alkenyl glycerols) have an alkyl chain of mostly 16 or 18 carbon atoms linked to the *sn*-1 position of the backbone glycerol by an ether bond. This ether bond, in contrast to the ester bond of the better described acyl lipids, provides this lipid class with a higher metabolic stability. Ether lipids are present in organisms ranging from bacteria, protozoa, fungi, higher plants to mammals including humans [1–3]. Most prevalent are these ether bonded side chains in phospholipids, here again saturated alkyl side chains are predominantly found in phosphatidylcholines and single unsaturated alkyl side chains (alk-1′-enyl) in phosphatidylethanolamines [4]. Alk-1′-enyl phospholipids have a vinyl ether bond and are termed plasmalogens. It has been shown that the amount of ether lipids in human and animal tumours is higher in neoplastic than in healthy cells [5,6]. Other studies have confirmed the increased presence of ether lipids in cancerous cells [7–10]. Encouraged by these findings, there were efforts trying to establish ether lipids as tumour markers in medical cancer diagnostics [11], however, as follow-up investigations found unaltered or even decreased amounts of ether lipids this path had to be abandoned [12,13].

Ether lipids in the diet are thought to have general beneficial effects on health. Shark oil which is especially rich in these lipids is used for wound healing and against gastric ulcers, colon inflammation and arthritis [14]. Ether lipids have been reported to be immunostimulatory by activation of macrophages and to confer an anti-angiogenic effect [15]. Their activity spectrum also includes antibacterial [16] and antifungal [17] properties and they can increase permeability of the blood brain barrier thereby enabling delivery of drugs to the brain [18–20]. Another study reported an increase in sperm motility in pigs and elevated fertility after treatment with these molecules [21]. In Madin–Darby canine kidney cells, alkylglycerols have been shown to inhibit protein kinase C (PKC) and thereby mediate cell-density dependence of proliferation *in vitro* [22].

A well-studied alkylglycerol is the platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, (*18*) in Fig. 1). It is involved in physiological processes such as e.g. inflammation, reproduction and blood pressure regulation. It has

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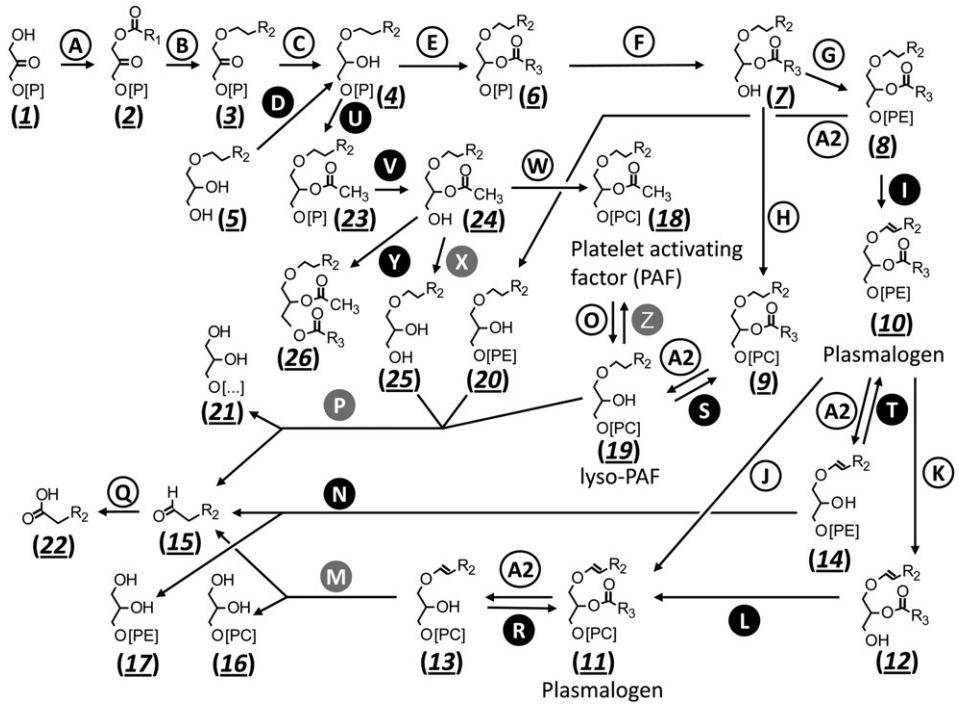

Fig. 1. Ether lipid metabolism including biosynthesis and degradation of PAF and plasmalogens. Reactions catalysed by orphan enzymes (i.e. enzymes with unknown sequence) are shown in black circles with white lettering and enzymes with recent sequence assignments in grey circles with white lettering. R, carbon side chain of R_1, mostly 15 or 17 atoms (=acyl); R_2, 14 or 16 atoms (=alkyl); R_3, at least 15 atoms, single- or poly-unsaturated (=acyl). For a description of the enzymatic reactions (capital letters) and the metabolites (Arabic numerals, underlined, italic) see text. ENZYMES (**A**) glycerone-phosphate *O*-acyltransferase (E.C. 2.3.1.42), (**A2**) phospholipase A2 (E.C. 3.1.1.4), (**B**) alkylglycerone-phosphate synthase (E.C. 2.5.1.26), (**C**) acylglycerone-phosphate reductase (E.C. 1.1.1.101), (**D**) alkylglycerol kinase (E.C. 2.7.1.93), (**E**) alkylglycerolphosphate 2-*O*-acyltransferase (E.C. 2.3.1.-), (**F**) phosphatidate phosphatase (E.C. 3.1.3.4), (**G**) ethanolamine-phosphotransferase (E.C. 2.7.8.1), (**H**) diacylglycerol cholinephosphotransferase (E.C. 2.7.8.2), (**I**) plasmanylethanolamine desaturase (E.C. 1.14.99.19), (**J**) a not further characterised transferase (E.C. 2.6.-.-), (**K**) phospholipase C (E.C. 3.1.4.3), (**L**) 1-alkylglycerophosphocholine *O*-acyltransferase (E.C. 2.7.8.22), (**M**) alkenylglycerophosphocholine hydrolase (E.C. 3.3.2.2), (**N**) alkenylglycerophosphoethanolamine hydrolase (E.C. 3.3.2.5), (**O**) 1-*O*-alkyl-2-acetylglycerophosphocholine esterase (E.C. 3.1.1.47), (**P**) alkylglycerol monooxygenase (E.C. 1.14.16.5), (**Q**) long-chain-aldehyde dehydrogenase (E.C. 1.2.1.48), (**R**) 1-alkenylglycerophosphocholine *O*-acyltransferase (E.C. 2.3.1.104), (**S**) 1-alkylglycerophosphocholine *O*-acyltransferase (E.C. 2.3.1.63), (**T**) 1-alkenylglycerophosphoethanolamine *O*-acyltransferase (E.C. 2.3.1.121), (**U**) alkylglycerophosphate 2-*O*-acetyltransferase (E.C. 2.3.1.105), (**V**) alkylacetylglycerophosphatase (E.C. 3.1.3.59), (**W**) diacylglycerol cholinephosphotransferase (E.C. 2.7.8.2), (**X**) acetylalkylglycerol acetylhydrolase (E.C. 3.1.1.71), (**Y**) 1-alkyl-2-acetylglycerol O-acyltransferase (E.C. 2.3.1.125), (**Z**) alkylglycerophosphocholine *O*-acetyltransferase (E.C. 2.3.1.67). METABOLITES (*1*) glycerone phosphate (dihydroxyacetone phosphate), (*2*) 1-acyl-glycerone 3-phosphate, (*3*) 1-*O*-alkyl-glycerone 3-phosphate, (*4*) 1-*O*-alkyl-*sn*-glycero-3-phosphate, (*5*) 1-*O*-alkyl-*sn*-glycerol, (*6*) 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphate, (*7*) 1-*O*-alkyl-2-acyl-*sn*-glycerol, (*8*) 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (plasmanylethanolamine), (*9*) 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (plasmanylcholine), (*10*) 1-*O*-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (plasmenylethanolamine), (*11*) 1-*O*-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine (plasmenylcholine), (*12*) 1-*O*-alkenyl-2-acyl-*sn*-glycerol, (*13*) 1-*O*-alk-1′-enyl-*sn*-glycero-3-phosphocholine (lysoplasmenylcholine), (*14*) 1-*O*-alk-1′-enyl-*sn*-glycero-3-phosphoethanolamine (lysoplasmenylethanolamine), (*15*) fatty aldehyde, (*16*) *sn*-glycero-3-phosphocholine, (*17*) *sn*-glycero-3-phosphoethanolamine, (*18*) 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF), (*19*) 1-*O*-alkyl-*sn*-glycero-3-phosphocholine (lyso-PAF), (*20*) 1-*O*-alkyl-*sn*-glycero-3-phosphoethanolamine, (*21*) glycerol or *sn*-glycero-3-phosphocholine or *sn*-glycero-3-phosphoethanolamine, (*22*) long-chain fatty acid, (*23*) 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphate, (*24*) 1-*O*-alkyl-2-acetyl-*sn*-glycerol, (*25*) 1-*O*-alkyl-*sn*-glycerol, (*26*) 1-*O*-alkyl-2-acetyl-3-acyl-*sn*-glycerol.

been reported to play a pathophysiological role in cardiovascular, renal, neuronal, pulmonary, immunological disorders and in shock [23] and was first described in 1972 [24]. PAF is synthesised by two alternative strategies in the body, the *de novo* and the remodelling pathway. For its role as mediator, the remodelling pathway is of prime importance. This pathway includes cleavage of a precursor lipid by phospholipase A2, acetylation at the *sn*-2 position to yield its active form, and deacetylation at the *sn*-2 position to inactivate the molecule (reactions (**A2**), (**Z**) and (**O**)), respectively, Fig. 1, [25].

Plasmalogens, a subclass of ether phospholipids which harbours a vinyl double bond in the alkyl side chain ((*10*) and (*11*) in Fig. 1), are found ubiquitously in animal cells and form 18% of the total phospholipid mass in humans [26]. The *sn*-2 position of glycerol in plasmalogens is very commonly acylated by poly-unsaturated fatty acids of the omega-3 and omega-6 class (e.g. arachidonic acid) [4], the *sn*-3 position carries either a phosphocholine or a phosphoethanolamine residue. Plasmalogens seem to have implications in protection against oxidative stress, however, their function still remains somewhat obscure (for review see [27]). Plasmalogen contents are highest in brain and spermatozoa and lowest in liver [4]. Another ether lipid species especially enriched in human spermatozoa is seminolipid, a sulfogalactolipid (for review see [28]).

Ether lipids are a constituent of glycosylphosphatidylinositol (GPI) anchors which link proteins to membranes via post-translational modification. GPI anchor synthesis has been shown to be indispensable for the germline development of the nematode *Caenorhabditis elegance* [29]. The lipid part of GPI anchors is frequently 1-*O*-alkyl-2-acyl-*sn*-glycerol.

1.2. Ether lipid metabolism

The biosynthetic routes to ether lipids including plasmalogens involve many integral membrane enzymes not all of which have been characterised in detail. However, most enzymatic steps are known. A short overview of the central part of ether lipid metabolism is given in Fig. 1, the respective enzyme names and their E.C. numbers can be found in Table 1. For more detailed information the reader is referred to a recent review [30].

The first step in ether lipid synthesis is catalysed by glycerone-phosphate *O*-acyltransferase (E.C. 2.3.1.42) (**A**), a peroxisomal enzyme that acylates glycerone phosphate (dihydroxyacetone