Role of Fatty Aldehyde Dehydrogenase in Ether Lipid Degradation Pathways

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Ether Lipid Metabolism
Ether-linked glycerolipids are present in most mammalian tissues including brain, kidney, heart and skin. They were once strictly referred to as structural components of membranes, but by now it is known that some are biologically active (1). Ether lipids have important roles as maintaining proper brain function, spermatogenesis, mediation of inflammation and lens organisation (2-4). The ether bond of ether lipids is synthesised by the action of two peroxisomal enzymes, dihydroxyacetonephosphate acyltransferase (DHAPAT) and alkyl-dihydroxyacetonephosphate acyltransferase (ADAPS) (5, 6). Lack of ether lipids in DHAPAT knockout mice causes early arrest of spermatogenesis, formation of a lenticulus anterior accompanied with the generation of cataract and hypomyelination in distinct regions of the central nerve system (reviewed by 2).

The sn-2 residue of ether lipids such as seminolipids, platelet-activating factor (PAF) and plasmaminelethanolamine is first cleaved off by specific hydrolases, or the keto group at C2 is reduced as depicted in figure 1. Then the resulting lyso-alkylglycerols are cleaved into a glycerol derivative and a long chain fatty aldehyde by alkylglycerol monoxygenase (glycerylether monoxygenase [EC 1.14.16.5]) in a tetrahydrobiopterin-dependent manner. Alkylglycerol monoxygenase is the only enzyme known to cleave the ether bond and accepts a wide range of ether lipids as substrates (reviewed by Watschinger et al., this issue).

Fatty Aldehyde Dehydrogenase
During cleavage of ether lipids by alkylglycerol monoxygenase, free fatty aldehydes are formed. Since these long chain fatty aldehydes are toxic to cells because of their high reactivity with free amino groups of lipids and proteins, the removal of these components is of great physiological importance (7). The enzyme fatty aldehyde dehydrogenase (FALDH [EC 1.2.1.48]) is responsible for the degradation of a wide variety of long and medium chain aldehydes (6 – 24 carbons, saturated, unsaturated, branched chain).
into their corresponding acids (8). This NAD\(^+\)-dependent enzyme is bound to microsomal and peroxisomal membranes by a single hydrophobic transmembrane domain which is located at the carboxy-terminus. As shown in figure 1, beside the degradation of alkylglycerols, fatty aldehydes are also produced in other lipid or fatty acid degradation pathways or by lipid-derived oxidative stress (9).

Based on a comparison of the crystal structure of a related class 3 aldehyde dehydrogenase with a sequence alignment to fatty aldehyde dehydrogenase, it was proposed that during the oxidation reaction fatty aldehydes are bound to a catalytically active cysteine residue of fatty aldehyde dehydrogenase via a thiohemiacetal, followed by a hydride transfer from the substrate to the NAD\(^+\) cofactor and hydrolysis of the fatty acid product (10).

**ALDH3A2 Gene**

Fatty aldehyde dehydrogenase is encoded by the ALDH3A2 gene. In humans, the gene is located on chromosome 17p11.2 and has a length of 28.8 kb (11). The mouse Aldh3a2 gene is mapped to chromosome 11 with a length of 22.4 kb (12). Fatty aldehyde dehydrogenase mRNA is widely distributed in mouse tissues. Highest mRNA expression levels are reached in liver, lung and intestinal tract. Lowest levels were found in muscle and heart (13).

The ALDH3A2 gene consists of 11 exons and 10 introns that can be spliced into two different variants (13). The major transcript (FALDHn) yields a 485 amino acid protein in man and accounts for about 90% of the total fatty aldehyde dehydrogenase expressed (exon 1-10). FALDHn is localised in the endoplasmatic reticulum. The less abundant splice variant (FALDHv) is 508 amino acids long and has an alternative exon 9’ spliced between exon 9 and 10. This leads to a modified carboxy-terminus (4 carboxy-terminal amino acids of fatty aldehyde dehydrogenase are replaced by 27 partly hydrophobic amino acids),

![Figure 1: Overview on the enzymatic steps required for ether lipid degradation](image)

In a first step the acyl or keto residue at the sn-2 position of ether lipids (like e.g. seminolipid, platelet-activating factor (PAF), plasmanylethanolamine or 1-alkyl glycerone-3-phosphate) is hydrolysed/reduced to a free hydroxyl group. The sn-1 ether bond of the emerging alkylglycerol species is then cleaved by the tetrahydrobiopterin-dependent enzyme alkylglycerol monooxygenase. In this reaction long chain fatty aldehydes are formed that are subsequently metabolised into their corresponding acids by fatty aldehyde dehydrogenase. Other lipid and fatty acid degradation pathways as well as oxidative stress are also sources for fatty aldehydes.
harbouring a peroxisomal targeting sequence and thereby directing the protein to the peroxisomal membranes (14).

Another aldehyde dehydrogenase, the cytosolic ALDH3A1, is only about 60 kb apart from ALDH3A2 on the human genome. Both genes consist of the same number of exons and their sequence is highly similar (66%), pointing to a duplication event. As zebrafish lacks an ALDH3A1 gene whereas ALDH3A2 is present it was suggested that ALDH3A2 is the ancestral gene (15).

Sjögren Larsson Syndrome (SLS)
Mutations in the ALDH3A2 gene can cause the Sjögren Larsson Syndrome (SLS, OMIM 270200, (16)), an autosomal recessive disorder that was first described by Sjögren and Larsson in 1957 (17). Symptoms of this disease such as ichthyosis, mental retardation and spastic di- and tetraplegia are caused by formation of Schiff base adducts of fatty aldehydes with free amino groups of lipids and proteins and by accumulation of fatty alcohols and wax esters in cells (18). SLS patients display only residual fatty aldehyde dehydrogenase activity ranging from 2 to 26% in patient skin fibroblasts when compared with healthy controls (19). Approximately 0.4 in 100,000 people worldwide suffer from this disorder (20).

So far more than 70 different Sjögren Larsson Syndrome causing mutations are known, including missense mutations, deletions, insertions and splicing errors. All mutations so far described are scattered throughout the whole gene with the exception of exons 9’ and 10 that are coding for the hydrophobic carboxyterminal tail where no mutations have been detected (21). Even though many of the other aldehyde dehydrogenases are well characterised, fatty aldehyde dehydrogenase is the first ALDH that causes fundamental neurological and dermal dysfunctions when mutated (16).

Determination of Fatty Aldehyde Dehydrogenase Activity
Measurement of fatty aldehyde dehydrogenase activity is used for the biochemical diagnosis of Sjögren Larsson Syndrome. There are different protocols for the quantification of fatty aldehyde dehydrogenase activity present in literature. In a common approach activity is quantified by the fluorometrical measurement of NADH (8), which is formed during the enzymatic reaction. Drawback of this method is mainly the influence of non-specific NADH forming reactions (enzymatic and chemical), which results in a high background activity and a reduced sensitivity of this method (19). Other groups use radiolabelled substrates to determine fatty aldehyde dehydrogenase activity, requiring laborious extraction and washing steps after termination of the enzymatic reaction (7). For activity measurement with GC-MS (as described by 22) or with ESI-MS (as described by 19) in addition to extraction and derivatisation steps, special instrumentation is needed which is not available in every laboratory. All these assays are performed in 200-500 µl total volume with 20-50 µg of cellular protein.

Novel Fatty Aldehyde Dehydrogenase Assay using the Fluorescent Substrate Pyrenedecanal
We developed a novel assay for the measurement of fatty aldehyde dehydrogenase activity by the use of the fluorescent labelled substrate pyrenedecanal (23). With isocratic reversed phase HPLC and fluorescent detection of the product pyrenedecanoic acid this method allows the reliable and sensitive determination of enzyme activity in nanogram amounts of microsomal or microgram amounts of fibroblast protein. Compared to other fatty aldehyde dehydrogenase assays, our novel assay features considerable advantages. On the one hand no extraction, washing or derivatisation steps are needed after termination of the enzymatic reaction, on the other hand the HPLC system that is required for separation and detection of the probe is readily available in many laboratories.

We are currently investigating the distribution of fatty aldehyde dehydrogenase activity in different mouse tissues. Additionally, we are interested in the differences of tissue specific fatty aldehyde dehydrogenase activity in a diabetic mouse model compared to wildtype mice.

Conclusion
During degradation of alkylglycerols by the tetrahydrobiopterin-dependent enzyme alkylglycerol monooxygenase long chain fatty aldehydes are formed. Since these aldehydes are
toxic to cells they are rapidly metabolised into fatty acids by NAD⁺-dependent fatty aldehyde dehydrogenase. Patients who suffer from Sjögren Larsen Syndrome, a genetic disorder that occurs when fatty aldehyde dehydrogenase activity is disturbed due to mutations in the corresponding gene, exhibit fundamental neurological and dermal dysfunctions.

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