

## Phytanic acid omega-oxidation in human liver microsomes and its implications for Refsum disease

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Patients with Refsum Disease (RD) have a deficiency in the degradation route for 3-methyl branched-chain fatty acids which is known as the  $\alpha$ -oxidation pathway (1). These fatty acids cannot be broken down by regular  $\beta$ -oxidation because of their 3-methyl group. During  $\alpha$ -oxidation the fatty acid is shortened by a one-carbon moiety to its n-1 analogue which is a substrate for  $\beta$ -oxidation because it now has the methyl-group on position 2. The main cause of RD are mutations in the gene coding for Phytanoyl-CoA Hydroxylase (PAHX) (2), the rate limiting enzyme of the pathway which is localized in peroxisomes. Furthermore, certain mutations in the *PEX7* gene which encodes the receptor involved in targeting PAHX to the peroxisome, also lead to RD (3).

RD is biochemically characterized by an accumulation of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) caused by deficient  $\alpha$ -oxidation. Phytanic acid is a highly abundant 3-methyl branched chain fatty acid. Its precursor, phytol, is part of the chlorophyll molecule and can be released from this molecule by the action of bacteria in the rumen of ruminant animals where it can be further converted into phytanic acid. Humans are not able to release phytol from chlorophyll, but are able to convert free phytol into phytanic acid (4). As a result, humans obtain phytanic acid and its precursor through the diet where it is highly present in meat and dairy products.

The accumulation of phytanic acid is believed to be the main cause of the pathology of RD. The symptoms include progressive night blindness leading to retinitis pigmentosa, peripheral neuropathy, and cerebellar ataxia (4;5). The only treatment currently known is a diet low in phytanic acid which may be combined with plasmapheresis. This treatment lowers the phytanic acid levels in RD patients which reduces the progression of the disease.

The decline in phytanic acid levels after treatment even in patients with a full block in the  $\alpha$ -oxidation pathway indicates that there is an alternative pathway capable of breaking down phytanic acid besides  $\alpha$ -oxidation. The third oxidation pathway for fatty acids, i.e. the  $\omega$ -oxidation pathway, is a candidate pathway for the alternative breakdown of phytanic acid. The

$\omega$ -oxidation pathway involves three enzymatic steps (Fig. 1). First, a cytochrome P450 (CYP450) hydroxylates phytanic acid at the  $\omega$ -end of the molecule. This is followed by the conversion of the  $\omega$ -hydroxylated fatty acid to an aldehyde by an alcohol dehydrogenase. Subsequently an aldehyde dehydrogenase converts the aldehyde into a dicarboxylic fatty acid, namely phytanedioic acid. Phytanedioic acid can further be degraded by  $\beta$ -oxidation from the  $\omega$ -end.

### Aim

Presumed metabolites of  $\beta$ -oxidized phytanedioic acid including 3-methyl adipic acid have been found elevated in RD patients (6) indicating that phytanic acid is actually a substrate for the  $\omega$ -oxidation pathway. The aim of our studies is to identify the enzymes involved in the  $\omega$ -oxidation pathway of phytanic acid. Our special interest is directed towards the first enzyme of the pathway, the CYP450, which is believed to catalyze the rate limiting step, i.e. the  $\omega$ -hydroxylation of phytanic acid. CYP450 enzymes form a large family of homologous proteins. Their expression can be induced by various known drugs, including fibrates, dexamethasone, and rifampicine. Upregulation of the specific CYP450 responsible for phytanic acid  $\omega$ -hydroxylation may lead to an increased flux of phytanic acid through the  $\omega$ -oxidation pathway. Consequently, the increased clearance of phytanic acid through the  $\omega$ -oxidation pathway may have obvious implications for the treatment of RD patients.

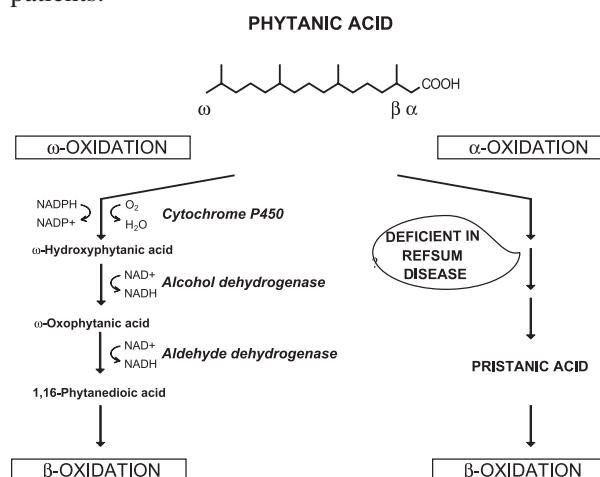


Figure 1. Phytanic acid degradation

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## Methods

The phytanic acid  $\omega$ -hydroxylation assay was performed according to the protocol described previously (7;8). In short, phytanic acid (200  $\mu$ M) was incubated with protein (1 mg/ml) (human or rat liver microsomes, or Supersomes<sup>TM</sup> (BD Gentest<sup>TM</sup>)) in a potassium phosphate buffer (0.1 M, pH 7.7) in the presence of methyl- $\beta$ -cyclodextrin (0.75 mg/ml). Reactions were initiated by addition of NADPH (1 mM) and were allowed to proceed for 30 minutes. The assay was terminated by the addition of HCl. Reaction products were extracted from the mixture with ethylacetate/diethylether (1:1) and derivatized with BSTFA 1% TMCS (v/v). Analysis of the products was done by GC/MS.

## Results

Our results show that  $\omega$ -hydroxylation of phytanic acid takes place under our assay conditions in both human and rat liver microsomes. Two products were identified from the corresponding mass spectrum, i.e.  $\omega$ -hydroxyphytanic acid and ( $\omega$ -1)-hydroxyphytanic acid. The ratio between  $\omega$ - and ( $\omega$ -1)-phytanic acid differed in human liver microsomes (15:1) as compared to rat liver microsomes. The optimal assay conditions are described in the methods section. Using these assay conditions with microsomes containing individually expressed recombinant CYP450 enzymes (Supersomes<sup>TM</sup>) it was found that some CYP450 enzymes of the family 4 class were able to  $\omega$ -hydroxylate phytanic acid in the following order of activity: CYP4F3A>CYP4F3B>CYP4A11>CYP4F2.

## Conclusions

From the results obtained in this study it can be concluded that phytanic acid indeed undergoes the first step of the  $\omega$ -oxidation pathway. The formation of  $\omega$ -hydroxyphytanic acid in human liver microsomes is far greater than ( $\omega$ -1)-hydroxyphytanic acid which is beneficial because the former product is a substrate for the next step in the pathway. CYP4F3A is the most active CYP450 but is not present in liver and therefore is not responsible for phytanic acid  $\omega$ -hydroxylation activity in liver. The other CYP450s

that have phytanic  $\omega$ -hydroxylation activity are present in liver. Currently we are studying whether the CYP450s involved in phytanic  $\omega$ -hydroxylation can be upregulated. In this respect it is important to mention that previous studies by other groups have shown that CYP4A11 is under control of the PPAR alpha nuclear hormone receptor and can be upregulated with fibrates (9). Unfortunately nothing is known about the upregulation of the more active CYP4F3 enzymes.

## Literature

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