Characterization of the human ω-oxidation pathway for ω-hydroxy-very-long-chain fatty acids

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ABSTRACT **Very-long-chain fatty acids (VLCFAs) have long been known to be degraded exclusively in peroxisomes *via* β-oxidation. A defect in peroxisomal β-oxidation results in elevated levels of VLCFAs and is associated with the most frequent inherited disorder of the central nervous system white matter, X-linked adrenoleukodystrophy. Recently, we demonstrated that VLCFAs can also undergo ω-oxidation, which may provide an alternative route for the breakdown of VLCFAs. The ω-oxidation of VLCFA is initiated by CYP4F2 and CYP4F3B, which produce ω-hydroxy-VLCFAs. In this article, we characterized the enzymes involved in the formation of very-long-chain dicarboxylic acids from ω-hydroxy-VLCFAs. We demonstrate that very-long-chain dicarboxylic acids are produced *via* two independent pathways. The first is mediated by an as yet unidentified, microsomal NAD^+-dependent alcohol dehydrogenase and fatty aldehyde dehydrogenase, which is encoded by the *ALDH3A2* gene and is deficient in patients with Sjögren-Larsson syndrome. The second pathway involves the NADPH-dependent hydroxylation of ω-hydroxy-VLCFAs by CYP4F2, CYP4F3B, or CYP4F3A. Enzyme kinetic studies show that oxidation of ω-hydroxy-VLCFAs occurs predominantly *via* the NAD^+-dependent route. Overall, our data demonstrate that in humans all enzymes are present for the complete conversion of VLCFAs to their corresponding very-long-chain dicarboxylic acids.—Sanders, R.-J., Ofman, R., Dacremont, G., Wanders, R. J. A., Kemp, S. Characterization of the human ω-oxidation pathway for ω-hydroxy-very-long-chain fatty acids. *FASEB J.* 22, 2064–2071 (2008)**

Key Words: X-linked adrenoleukodystrophy · cytochrome P450 · Sjögren-Larson syndrome · ALDH3A2

X-LINKED ADRENOLEUKODYSTROPHY (X-ALD; OMIM 300100) is the most common inherited peroxisomal disorder with an incidence of ~1 in 17,000 (1). It is a progressive neurodegenerative disease that affects cerebral white matter, spinal cord, peripheral nerves, adrenal cortex, and testis (2). X-ALD is caused by mutations in the *ABCD1* gene, which encodes the adrenoleukodystrophy protein (ALDP), an ATP-binding cassette transporter protein located in the peroxisomal membrane (3). One of the primary peroxisomal functions is detoxification of very-long-chain fatty acids (VLCFAs) (>22 carbons) by chain shortening *via* β-oxidation (4). Although the exact role of ALDP remains elusive, it has been implicated in the transport of VLCFA into peroxisomes (5, 6). In patients with X-ALD, the defect in ALDP results in decreased β-oxidation of VLCFAs (7). As a result, VLCFAs accumulate in all tissues and plasma (8). In brain, ALDP is expressed in all glial cell types, including astrocytes, microglial cells, and oligodendrocytes (9). Myelinated tracts of oligodendrocytes have a high lipid turnover. Therefore, incorporation of VLCFAs, especially C26:0, in components of the multilamellar myelin membrane might lead to destabilization (10, 11). X-ALD is associated with increased levels of VLCFAs. Hence, correction of VLCFA levels is the primary objective in many therapeutic approaches, including lovastatin treatment (12), dietary treatment with Lorenzo's oil (13), or induction of the expression of the ALDP-related protein (14). The aim of other interventions, such as gene replacement therapy (15) and bone marrow transplantation (16), is to prevent the incipient cerebral inflammatory demyelination by replacing the malfunction gene.

Recently, we demonstrated that VLCFAs can also be oxidized *via* another mechanism, *i.e.*, ω-oxidation (17). The first step in this pathway involves the hydroxylation of the ω-methyl group of the VLCFA, generating a ω-hydroxy-VLCFA. This reaction requires NADPH and molecular oxygen as cofactors and is catalyzed by two cytochrome P450 (CYP450) enzymes belonging to the CYP4F subfamily, namely CYP4F2 and CYP4F3B (17). Subsequently, the ω-hydroxy-VLCFA is oxidized further into the corresponding dicarboxylic acid either *via* a CYP450-mediated route or *via* an as yet unidentified NAD^+-dependent pathway (17–21). Long-chain dicarboxylic acids are β-oxidized in peroxisomes to shorter-

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chain dicarboxylic acids followed by excretion into the urine (22). Studies with fibroblasts from patients with X-ALD have demonstrated that β-oxidation of long-chain dicarboxylic acids is normal, in contrast to fibroblasts from patients with a peroxisomal biogenesis disorder (22). These studies indicate that peroxisomes are essential for the metabolism of dicarboxylic acids and that this process does not require ALDP. Moreover, patients with a peroxisomal biogenesis disorder excrete considerable amounts of long-chain dicarboxylic acids in urine (23). Stimulation of VLCFA ω-oxidation could provide an interesting option to reduce VLCFA levels in patients with X-ALD. It should be noted, however, that as yet no data are available with respect to the β-oxidation capacity of peroxisomal very-long-chain dicarboxylic acids.

The enzymes involved in the conversion of ω-hydroxy-VLCFA into their dicarboxylic acids have not been identified. This information is of pivotal importance for further investigations to determine a therapeutic approach. In this article we have identified and characterized the enzymes involved in the ω-oxidation of VLCFAs in human microsomes.

MATERIALS AND METHODS

Chemicals

22-Hydroxydocosanoic acid (ω-hydroxy-C22:0) and hexacosanedioic acid (C26:0-DCA) were purchased from Larodan Fine Chemicals (Malmö, Sweden). A stock solution of 2.5 mM was prepared in dimethyl sulfoxide. Disulfiram and 17-octadecynoic acid (17-ODYA) were from Sigma-Aldrich Corp. (St. Louis, MO, USA). NAD^+ and NADPH were from Roche Applied Science (Almere, The Netherlands). Pooled human liver S9, cytosol, and microsomes and recombinant human CYP450-containing insect cell microsomes (Supersomes) were from BD Biosciences/Gentest (Woburn, MA). The CYP450 contents of Supersomes as provided by the manufacturer were CYP2E1 (588 pmol/mg), CYP2J2 (185 pmol/mg), CYP3A4 (606 pmol/mg), CYP4A11 (120 pmol/mg), CYP4F2 (556 pmol/mg), CYP4F3A (33 pmol/mg), CYP4F3B (435 pmol/mg), and CYP4 F12 (213 pmol/mg). All other chemicals used were of analytical grade.

Enzymatic ω-oxidation assays

The experimental conditions to study the ω-oxidation of ω-hydroxy fatty acids were the same as those described previously (17, 20) with minor modifications, as discussed below.

NAD^+-dependent ω-oxidation assay

The reaction mixture contained glycine (100 mM); HEPES, pH 9.7 (100 mM); protein (200 or 50 μg/ml for ω-hydroxy-C22:0 and ω-hydroxy-C26:0, respectively); α-cyclodextrin (1 mg/ml); and NAD^+ (1 mM) in a total volume of 200 μl.

NADPH-dependent ω-oxidation assay

The reaction mixture contained Tris buffer, pH 8.4 (100 mM), protein (50 μg/ml), α-cyclodextrin (1 mg/ml), and NADPH (1 mM) in a total volume of 200 μl. The reactions were initiated by addition of the ω-hydroxy fatty acid at a final concentration of 100 μM and were allowed to proceed for 30 min at 37°C. After termination of the reactions by the addition of 1 ml of hydrochloric acid (2 M), the reaction products were extracted as described previously and analyzed by electrospray ionization tandem mass spectrometry (24).

Tissue culture conditions

Fibroblasts were cultured in nutrient Ham's F-10 medium supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C under 5% CO_2. Primary human fibroblast cell lines were obtained from normal control subjects and patients with Sjögren-Larsson syndrome (SLS). In each patient, the diagnosis was established using clinical, biochemical, and molecular methods, which included demonstration of deficient fatty aldehyde dehydrogenase (FALDH) activity and the identification of mutations in the *ALDH3A2* gene (25). Cells were harvested with trypsin and washed twice with PBS and once with 0.9% NaCl. Cells were taken up in ice-cold PBS and homogenized by sonication (two cycles of 10 s at 8 W) on ice, and the protein concentration was determined using the bicinchoninic acid method (28).

Subcellular fractionation studies

A liver biopsy sample was taken from a patient undergoing abdominal surgery with informed consent from the patient to use this material for scientific research. Approximately 1 g of liver was washed and homogenized on ice, using a glass Potter tube with a Teflon pestle, in 20 ml of ice-cold solution containing 250 mM sucrose, 2.5 mM EDTA, and 5 mM morpholinepropanesulfonic acid (MOPS) at pH 7.4. A postnuclear supernatant was obtained by centrifugation at 600 *g* for 10 min at 4°C. An organellar pellet was prepared by centrifugation at 12,000 *g* for 20 min at 4°C and used for density gradient centrifugation as described (26). Marker enzymes were measured to localize the different subcellular compartments in the gradient, as described elsewhere (27). Protein concentrations were determined using the bicinchoninic acid method (28).

RESULTS

Characterization of ω-hydroxy-VLCFA oxidation

To study the conversion of ω-hydroxy fatty acids into their corresponding dicarboxylic acids, the assay was optimized for ω-hydroxy-C22:0 using a postnuclear supernatant prepared from human liver. The production of C22:0-DCA was measured as a function of time, substrate concentration, and pH either with NAD^+ or with NADPH as cofactor (**Fig. 1**). Formation of C22:0-DCA in the presence of NAD^+ was optimal at pH 9.7 (Fig. 1*A*), and at this pH product formation was linear with time up to 60 min (Fig. 1*B*). The enzyme activity as

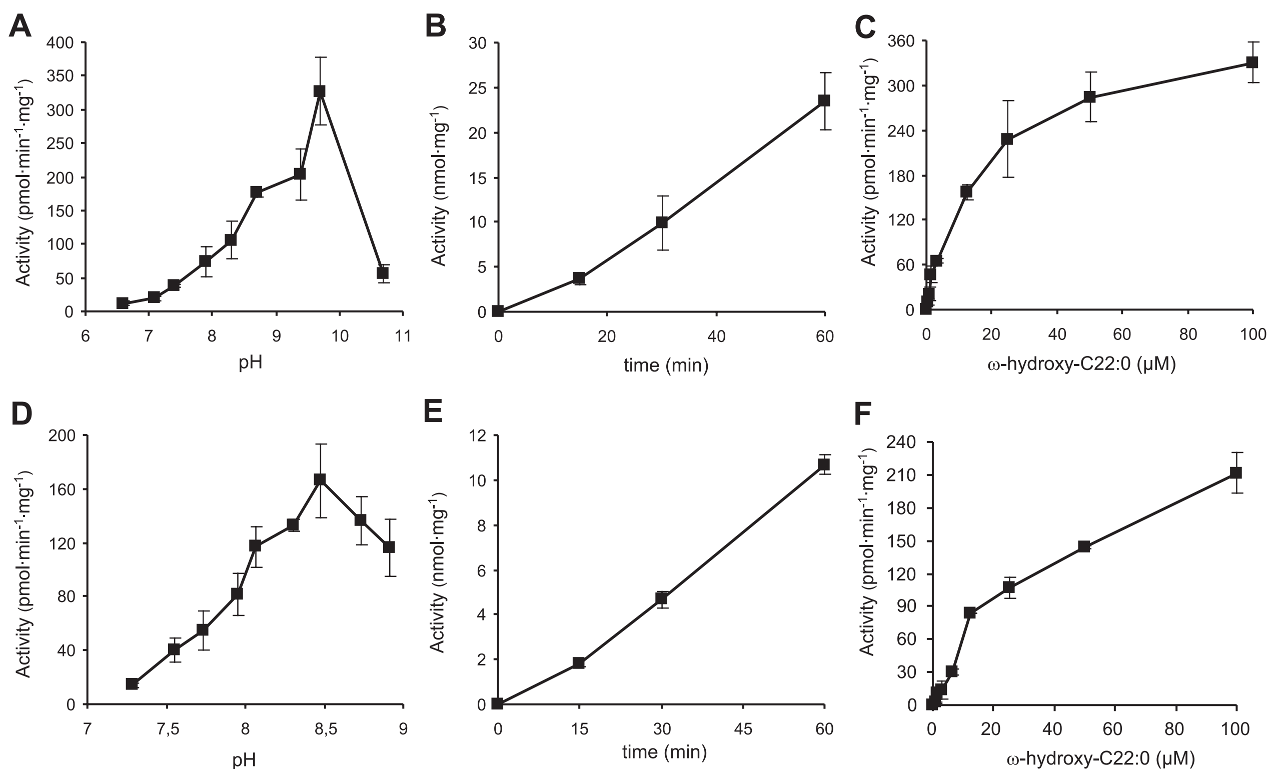

Figure 1. Optimization of the ω-oxidation activity assay in human liver. For NAD^+-dependent ω-oxidation, protein samples were incubated in a reaction mixture containing 100 mM glycine, 100 mM HEPES, 1 mM NAD^+, α-cyclodextrin, and ω-hydroxy-C22:0. Production of C22:0-DCA was measured as a function of pH (*A*), time (*B*), and ω-hydroxy-C22:0 concentration (*C*). For NADPH-dependent ω-oxidation, reactions were performed in a reaction mixture containing 100 mM Tris · HCl buffer (pH 8.4) and 1 mM NADPH. Formation of C22:0-DCA was determined as a function of pH (*D*), time (*E*), and ω-hydroxy-C22:0 concentration (*F*). The results are the means of three independent experiments; error bars represent the SD.

a function of substrate concentration (Fig. 1*C*) was used to calculate the kinetic constants K_m and V_{max} (**Table 1**).

With NADPH as cofactor, maximal activity was found at pH 8.4 (Fig. 1*D*). At this pH, activity was linear up to 60 min (Fig. 1*E*). Again, the enzyme activity as a function of substrate concentration (Fig. 1*F*) was used to calculate the kinetic constants K_m and V_{max} (Table 1). The optimal pH for the NAD^+- and NADPH-dependent ω-oxidation routes were similar to the previously observed pH optima in rat liver microsomes (20). These optimal conditions were used to determine the enzyme kinetic parameters for the two pathways with both ω-hydroxy-C22:0 and ω-hydroxy-C26:0 using a postnuclear supernatant fraction as the source of enzyme (Table 1). Both ω-hydroxy-fatty acids were substrates for the NAD^+-dependent oxidation route as well as for the NADPH-mediated pathway. Remarkably, the apparent affinity (K_m) as well as the catalytic efficiency (V_{max}/K_m) were substantially higher with the ω-hydroxy-C26:0 as substrate compared with ω-hydroxy-C22:0, independent of whether the NAD^+-dependent or the NADPH-dependent pathway was studied.

TABLE 1. *NAD^+-dependent and NADPH-dependent ω-oxidation activity in different human liver subcellular fractions*

	ω-Hydroxy-C22:0			ω-Hydroxy-C26:0		
	K_m^{app} (μM)	V_{max} (pmol min^{-1} mg^{-1})	V_{max}/K_m	K_m^{app} (μM)	V_{max} (pmol min^{-1} mg^{-1})	V_{max}/K_m
NAD^+-dependent						
S9	11.6	341.9	29.4	2.7	138.4	51.3
Cytosol	10.8	44.7	4.1	8.0	33.5	4.2
Microsomes	11.8	628.5	53.3	1.2	219.0	182.5
NADPH-dependent						
S9	21.5	147.5	6.9	3.0	66	22
Microsomes	20.8	568.6	27.3	3.8	256.5	69.9

Apparent K_m (K_m^{app}) and V_{max} were calculated from Michaelis-Menten plots derived from two individual experiments in which ω-hydroxy-C22:0 or ω-hydroxy-C26:0 was added at final concentrations ranging from 0.4 to 100 μM.

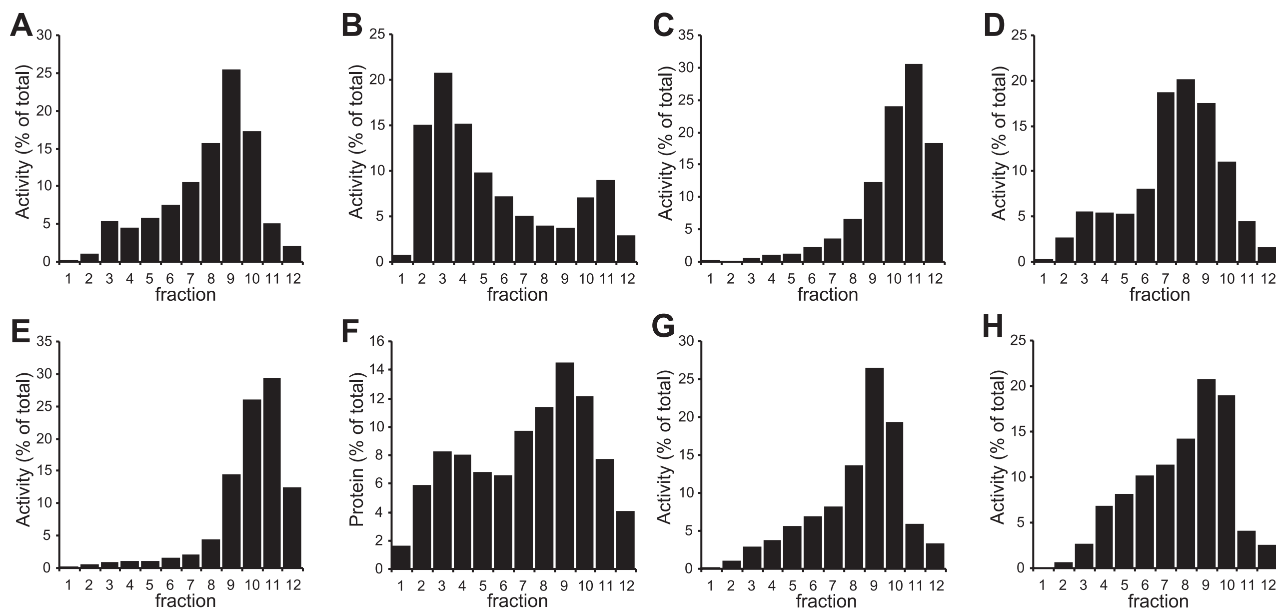

Figure 2. Subcellular localization of NAD^+-dependent ω-oxidation activity in a human liver Nycodenz density gradient. To localize the different organelles in the gradient, the following marker enzymes were measured: esterase (microsomes) (*A*), catalase (peroxisomes) (*B*), β-hexosaminidase (lysosomes) (*C*), glutamate dehydrogenase (mitochondria) (*D*), phosphoglucoisomerase (cytosol) (*E*), protein (*F*), ω-hydroxy-C22:0 NAD^+-dependent activity (*G*), and ω-hydroxy-C26:0 NAD^+-dependent activity (*H*). All enzyme activities are expressed as the percentage of total activity, and protein concentrations are expressed as the percentage of total protein. The results are the mean of two independent experiments.

Subcellular localization of the NAD^+-dependent ω-oxidation activity

To determine the subcellular localization of the conversion of ω-hydroxy-VLCFAs into ω-carboxy-VLCFAs in more detail, we measured the activity in fractions of a human liver density gradient with NAD^+ present. The results depicted in **Fig. 2** show that the activity profile observed with both ω-hydroxy-C22:0 (Fig. 2*G*) and with ω-hydroxy-C26:0 (Fig. 2*H*) closely mimics that of the microsomal marker esterase (Fig. 2*A*). This finding demonstrates that the NAD^+-dependent conversion of ω-hydroxy-VLCFAs in human liver is associated with the microsomal fraction.

Inhibition studies on NAD^+-dependent dehydrogenase activity

As ω-oxidation of ω-hydroxy-VLCFAs is predominantly associated with microsomes, we tested whether disulfiram, which is a potent inhibitor of human microsomal FALDH (28), also inhibits this activity, at least in the presence of NAD^+. **Figure 3** shows that the production of the dicarboxylic acid of C22:0 decreased with increasing disulfiram concentrations. Interestingly, the decrease of C22:0-DCA was accompanied by an increase of a peak at m/z value 353. On the basis of the calculated m/z value this may represent the intermediate metabolite of ω-hydroxy-C22:0, *i.e.*, the ω-aldehyde of C22:0 (ω-oxo-C22:0). Unfortunately, ω-oxo-C22:0 is not commercially available and is difficult to synthesize, so that unequivocal evidence for the identity of ω-oxo-C22:0 could not be obtained.

ω-Oxidation of ω-hydroxy-C22:0 in FALDH-deficient fibroblasts

To provide conclusive evidence for a role of FALDH in the conversion of ω-hydroxy-VLCFAs into the corresponding dicarboxylic acids, fibroblasts from control

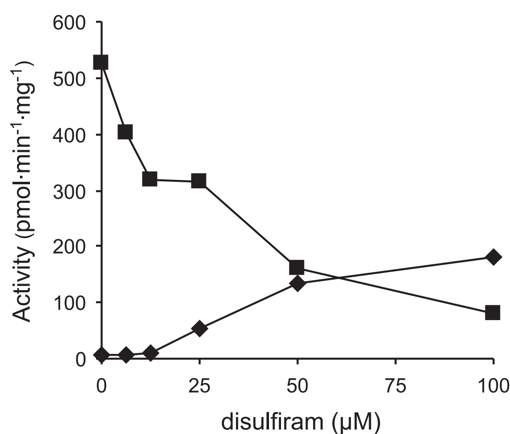

Figure 3. Effect of disulfiram on the NAD^+-dependent ω-oxidation activity of ω-hydroxy-C22:0. Protein samples were preincubated for 10 min in the standard reaction mixture with different concentrations of disulfiram. After preincubation, ω-hydroxy-C22:0 was added to a final concentration of 100 μM. All data shown represent the means of two independent experiments. ♦, ω-oxo-C22:0; ■, C22:0-DCA.

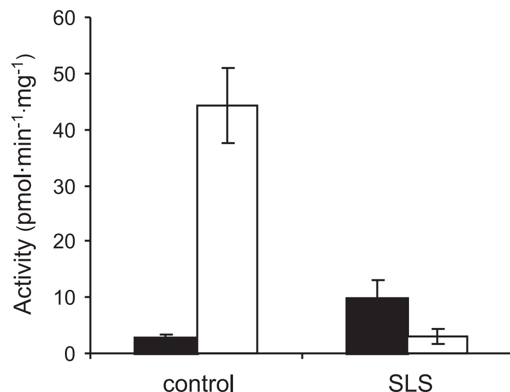

Figure 4. NAD$^+$-dependent ω-oxidation activity of ω-hydroxy-C22:0 in human skin fibroblasts. The activity was measured in fibroblast homogenates from control subjects (n=3) and patients with SLS (n=3) as described. ■, specific activity of the production of ω-oxo-C22:0; □, specific activity of the production of C22:0-DCA. Error bars represent the SD.

subjects and patients with SLS were assayed for ω-oxidation activity of ω-hydroxy-C22:0. The results depicted in **Fig. 4** show that the production of C22:0-DCA from ω-hydroxy-C22:0 is markedly decreased in fibroblasts from patients with SLS compared with control subjects. Interestingly, the presumed ω-oxo-C22:0 intermediate was found to accumulate to much higher levels in the incubations with homogenates of fibroblasts from patients with SLS compared with homogenates from control fibroblasts.

Identification of CYP450 ω-hydroxy fatty acid hydroxylases

Because ω-hydroxy-VLCFAs can also be metabolized by cytochrome P450 enzymes in the NADPH-driven ω-oxidation pathway, we investigated the effect of 17-ODYA on enzyme activity (20). Previously, we demonstrated that the NADPH and molecular oxygen-dependent conversion of ω-hydroxy-C22:0 was inhibited markedly by 17-ODYA, at least in rat liver microsomes (20). 17-ODYA is a specific inhibitor of CYP450 enzymes belonging to the CYP4 family (29, 30). To determine whether these enzymes are also involved in the ω-oxidation of ω-hydroxy-VLCFAs in human, we studied the effect of 17-ODYA on the production of C22:0-DCA and C26:0-DCA in human liver microsomes. **Figure 5** shows that the formation of dicarboxylic acids either with ω-hydroxy-C22:0 or with ω-hydroxy-C26:0 as substrate was reduced strongly by 17-ODYA, with an IC_{50} of ~0.8 μM. Finally, microsomes from insect cells expressing recombinant human CYP450 enzymes (Supersomes) were used to investigate which human CYP450 enzymes were reactive with ω-hydroxy-C22:0 and ω-hydroxy-C26:0. The data in **Fig. 6** show that four of the human recombinant CYP450 enzymes that were tested, CYP4F2, CYP4F3A, CYP4F3B, and CYP4A11, can use ω-hydroxy-C22:0 as substrate. However, only CYP4F2 and CYP4F3B show activity toward ω-hydroxy-C26:0.

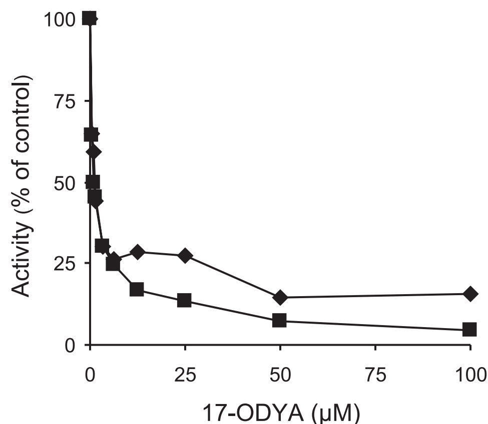

Figure 5. Effect of 17-ODYA on the NADPH-dependent CYP450-mediated ω-hydroxylation of ω-hydroxy-C22:0 (■) and ω-hydroxy-C26:0 (♦). Protein samples were preincubated for 10 min in the standard reaction mixture with different concentrations of 17-ODYA. After preincubation, the substrate was added to a final concentration of 100 μM. The results represent the mean of two independent experiments.

At optimal reaction conditions, the enzyme kinetic parameters for the individually expressed CYP450 enzymes were determined using different substrates (**Table 2**). CYP4F2 and CYP4F3B did show some enzymatic activity with ω-hydroxy-C22:0 and ω-hydroxy-C26:0 as substrates, although the apparent K_m values were considerably higher compared with the NAD$^+$-dependent oxidation route in human liver postnuclear supernatant fractions (Table 1). In contrast, CYP4F3A displayed a high affinity for ω-hydroxy-C22:0 (0.6 μM), whereas

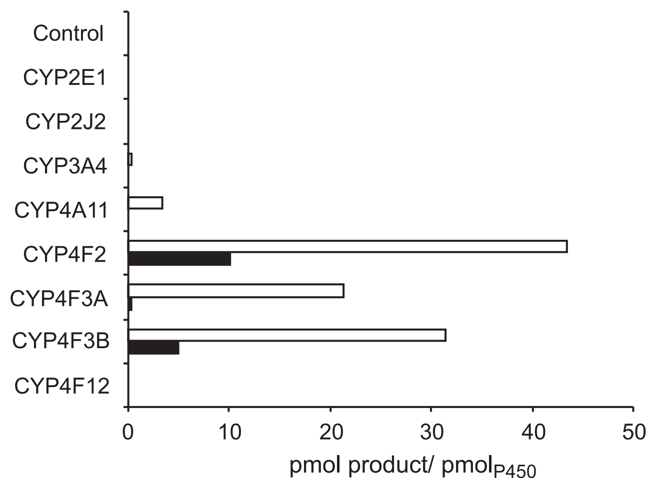

Figure 6. Identification of cytochrome P450 enzymes involved in the NADPH-dependent conversion of ω-hydroxy-VLCFAs into dicarboxylic acids. □, specific activity of human recombinant CYP450 enzymes with ω-hydroxy-C22:0; ■, specific activity of human recombinant CYP450 enzymes with ω-hydroxy-C26:0. Individual recombinant CYP450 proteins (5 pmol) were incubated for 30 min in the standard CYP450 ω-oxidation reaction mixture at 37°C. All data shown represent the means of two independent experiments, which did not vary by >10%.

TABLE 2. *Enzymatic characterization of recombinant human P450 enzymes*

	ω-Hydroxy-C22:0			ω-Hydroxy-C26:0		
	K_m^{app} (μM)	V_{max} (pmol min^{-1} pmol P450^{-1})	V_{max}/K_m	K_m^{app} (μM)	V_{max} (pmol min^{-1} mg^{-1})	V_{max}/K_m
CYP4F2	37.6	1.6	0.04	8.8	0.7	0.08
CYP4F3A	0.6	0.2	0.33	ND	ND	ND
CYP4F3B	13.1	0.3	0.02	5.2	0.8	0.15

Apparent K_m (K_m^{app}) and V_{max} were calculated from Michaelis-Menten plots derived from two individual experiments in which ω-hydroxy-C22:0 or ω-hydroxy-C26:0 was added at final concentrations ranging from 0.4 to 100 μM. ND, not detectable.

activity was below the limit of detection with longer chain ω-hydroxy-VLCFAs such as ω-hydroxy-C26:0. Overall, these data suggest that the oxidation of ω-hydroxy-fatty acids occurs predominantly *via* the NAD^+-dependent route.

DISCUSSION

In this article we have studied the enzymatic conversion of ω-hydroxy-VLCFAs into dicarboxylic acids in human liver. Previously, we demonstrated that CYP4F2 and CYP4F3B are involved in the NADPH-dependent ω-hydroxylation of VLCFAs in human liver (17). Although the main product of these CYP450 enzymes is ω-hydroxy-VLCFAs, we also observed some ω-carboxy-VLCFAs being produced. Our studies using rat liver microsomes revealed that the formation of ω-carboxy-C22:0 from ω-hydroxy-C22:0 may follow two distinct pathways, the first driven by NADPH and the second by NAD^+ (20). To elucidate whether these two pathways exist in humans, enzyme assays were set up for both the NAD^+-dependent and the NADPH-dependent pathways. Experiments with the individually expressed CYP450 enzymes showed that CYP4F2, CYP4F3A, CYP4F3B, and CYP4A11 displayed affinity toward ω-hydroxy-C22:0 whereas only CYP4F2 and CYP4F3B were found to be active with ω-hydroxy-C26:0. Analysis of the enzyme characteristics revealed that the NAD^+-dependent pathway has the highest catalytic efficiency (V_{max}/K_m) for both ω-hydroxy-C22:0 and ω-hydroxy-C26:0 compared with the NADPH-dependent route (Table 1). This finding suggests that the NAD^+-dependent pathway is most likely the major route, at least in liver. Subcellular localization studies in human liver indicated that the NAD^+-dependent conversion of both ω-hydroxy-C22:0 and ω-hydroxy-C26:0 is associated predominantly with the microsomes. The NAD^+-dependent conversion of ω-hydroxy-VLCFAs into its ω-carboxy-VLCFAs needs the concerted action of an alcohol dehydrogenase and an aldehyde dehydrogenase. At present, 19 distinct aldehyde dehydrogenase genes have been identified in the human genome (31). At least three ALDHs have been localized to the endoplasmic reticulum and are encoded by the *ALDH3B1*, *ALDH3B2*, and *ALDH3A2* genes (32–34). *ALDH3B1* is expressed mainly in kidney and lung and *ALDH3B2* is expressed in the parotid glands. Microsomal FALDH encoded by the *ALDH3A2* gene is expressed predominantly in liver, heart, and muscle (35). FALDH catalyzes the NAD^+-dependent oxidation of saturated and unsaturated aliphatic aldehydes ranging from 6 to 24 carbons, with an optimal preference for substrates with 16 to 20 carbon atoms. In addition, FALDH interacts with fatty alcohol dehydrogenase to form a fatty alcohol:NAD^+ oxidoreductase enzyme complex that catalyzes the overall conversion of fatty alcohols to fatty acids (36). Both FALDH (35), and the NAD^+-dependent conversion of ω-hydroxy-C22:0 are highly sensitive toward disulfiram (Fig. 3), indicating that FALDH is involved in this reaction. It has been reported previously that the human and rat microsomal aldehyde dehydrogenases have different sensitivity toward disulfiram (37, 38). This difference could explain the discrepancy between the results described in this article and our previous studies on the NAD^+-dependent dehydrogenation of ω-hydroxy-C22:0 in rat liver microsomes (20). However, conclusive evidence was derived from studies with fibroblasts from patients with SLS in which FALDH is deficient owing to mutations in the *ALDH3A2* gene (25). These experiments showed that FALDH indeed is involved in the conversion of ω-oxo-C22:0 into the corresponding dicarboxylic acid. In incubations with SLS fibroblast homogenates, the amount of ω-carboxy-C22:0 was significantly reduced compared with that in control fibroblasts. In addition, SLS fibroblasts accumulated the presumed ω-oxo-C22:0 intermediate. It has been suggested that FALDH forms a complex with a fatty alcohol dehydrogenase and that the fatty alcohol dehydrogenase activity is the rate-limiting step in the oxidation of fatty alcohols to fatty acids (39). Our data suggest that there is some residual alcohol dehydrogenase activity in the SLS fibroblasts used.

To summarize, the human ω-oxidation system can use VLCFAs for complete conversion into dicarboxylic acids. The first rate-limiting step (**Fig. 7*A***) is catalyzed by cytochrome P450 enzymes belonging to the CYP4F subfamily, *i.e.*, CYP4F2 and CYP4F3B (17). Further oxidation can occur *via* two distinct routes, including the NAD^+-dependent oxidation route *via* an as yet unidentified alcohol dehydrogenase and FALDH (Fig. 7*B, C*) or the NADPH-dependent route, which may involve CYP4F2, CYP4F3A, CYP4F3B, and CYP4A11

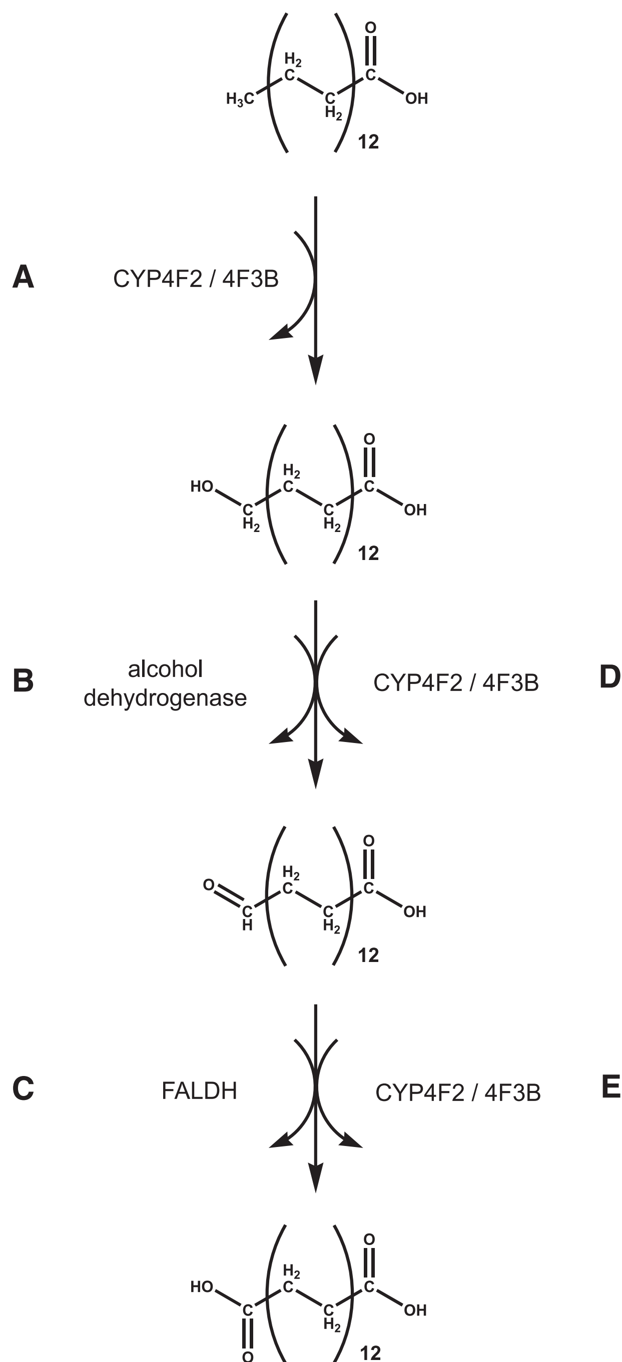

Figure 7. Human ω-oxidation routes for C26:0. See Discussion section for more details.

(Fig. 7*D, E*). Stimulation of VLCFA ω-oxidation by increased expression of CYP4F2 and CYP4F3B would affect not only the first reaction step of this pathway (Fig. 7*A*) but also the subsequent reaction steps. Recently, it has been shown that statins, *i.e.*, lovastatin, are able to induce the expression of the CYP4F2 gene *via* sterol regulatory element-binding proteins (40). Although there are conflicting data in the literature considering statin treatment in patients with X-ALD, early treatment with lovastatin may be beneficial for patients with X-ALD through elevated expression of CYP4F2 (14). We have established that the enzymatic pathway necessary to convert VLCFAs to their corresponding very-long-chain dicarboxylic acids is present in humans. The aim of future experiments will be to resolve whether induction of VLCFA ω-oxidation results in normalization of VLCFA levels in X-ALD.

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