

Phytanic acid α -oxidation: decarboxylation of 2-hydroxyphytanoyl-CoA to pristanic acid in human liver

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Abstract The degradation of the first intermediate in the α -oxidation of phytanic acid, 2-hydroxyphytanoyl-CoA, was investigated. Human liver homogenates were incubated with 2-hydroxyphytanoyl-CoA or 2-hydroxyphytanoyl-CoA, after which formation of 2-ketophytanic acid and pristanic acid were studied. 2-Hydroxyphytanoyl-CoA was converted into 2-ketophytanic acid and pristanic acid. When ATP, Mg²⁺, and coenzyme A were added to the incubation medium, higher amounts of pristanic acid were formed, whereas the formation of 2-ketophytanic acid strongly decreased. When 2-hydroxyphytanoyl-CoA was used as substrate, there was virtually no 2-ketophytanic acid formation. However, pristanic acid was formed in higher amounts than with 2-hydroxyphytanoyl-CoA as substrate. This reaction was stimulated by NAD⁺ and NADP⁺. Pristanic acid, and not pristanoyl-CoA was found to be the product of the reaction. These results suggest the existence of two pathways for decarboxylation of 2-hydroxyphytanoyl-CoA. The first one, starting from 2-hydroxyphytanoyl-CoA, involves the formation of 2-ketophytanic acid with only a small amount of pristanic acid being formed. The second pathway, which starts from 2-hydroxyphytanoyl-CoA, does not involve 2-ketophytanic acid and generates higher amounts of pristanic acid. The first pathway, which is peroxisomally localized, was found to be deficient in Zellweger syndrome, whereas the second pathway, localized in microsomes, was normally active. We conclude that the second pathway is predominant under in vivo conditions.—**Verhoeven, N. M., R. J. A. Wanders, D. S. M. Schor, G. A. Jansen, and C. Jakobs.** Phytanic acid α -oxidation: decarboxylation of 2-hydroxyphytanoyl-CoA to pristanic acid in human liver. *J. Lipid Res.* 1997. **38:** 2062–2070.

Supplementary key words gas chromatography–mass spectrometry • peroxisome • microsome

The interest in phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) dates back to the early sixties, when it was found that accumulation of this fatty acid was the biochemical abnormality in Refsum disease (1). Through the years, more diseases in which phytanic

acid accumulates, in combination with other metabolites, were reported. In generalized peroxisomal disorders, like Zellweger syndrome, phytanic acid accumulates next to other compounds that are normally metabolized in peroxisomes (2). In rhizomelic chondrodysplasia punctata (RCDP), phytanic acid accumulation is found in combination with impaired plasmalogen synthesis and impaired processing of the peroxisomal 3-ketoacyl-CoA thiolase; whereas the other peroxisomal functions are normal (2).

The mechanism and subcellular localization of phytanic acid α -oxidation have long been debated and remain incompletely understood. It is known that, due to its methyl group in the β -position, phytanic acid is first subjected to one cycle of α -oxidation. The pristanic acid that results can further be oxidized by β -oxidation, a peroxisomal process (3). Recently, it was found that phytanic acid is activated to phytanoyl-CoA prior to conversion into 2-hydroxyphytanoyl-CoA (4). The enzyme involved in the latter step, phytanoyl-CoA hydroxylase, is a dioxygenase type of enzyme, using Fe²⁺ and ascorbate as cofactors and 2-ketoglutarate as cosubstrate. It was found to be localized in peroxisomes and deficient in classical Refsum disease (5), in Zellweger syndrome (6), and in RCDP (7).

The metabolic pathway from 2-hydroxyphytanoyl-CoA to pristanic acid (or pristanoyl-CoA) is still unclear. It is known that 2-ketophytanic acid can be formed from 2-hydroxyphytanoyl-CoA in vitro (8). How-

Abbreviations: MOPS, morpholino-propanesulfonic acid; GC–MS, gas chromatography–mass spectrometry; RCDP, rhizomelic chondrodysplasia punctata.

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ever, 2-ketophytanic acid could never be demonstrated in human plasma (9) and its role in the phytanic acid α -oxidation pathway is disputed.

In this paper, we have studied the metabolic fate of 2-hydroxyphytanoyl-CoA, the product of the phytanoyl-CoA hydroxylase reaction. Our results show that 2-hydroxyphytanoyl-CoA is decarboxylated to pristanic acid in an NAD⁺- or NADP⁺-dependent reaction. This reaction is localized in microsomes and is normally active in livers obtained from Zellweger patients.

EXPERIMENTAL PROCEDURES

Materials

2-Hydroxyphytanic acid, 2-ketophytanic acid ethyl ester, [3-methyl-²H₃]-2-ketophytanic acid ethyl ester, pristanic acid, and [2-methyl-²H₃]pristanic acid were synthesized as described before (9, 10). 2,3-Pristenic acid and [2-methyl-²H₃]2,3-pristenic acid were synthesized as will be described elsewhere. The chemical purity of the compounds was >95%, the isotopic purity >98%.

2-Hydroxyphytanoyl-CoA was synthesized from 2-hydroxyphytanic acid using the acid anhydride method (11).

Patients

The patients whose liver samples were studied in the experiments described in this paper showed all the clinical and biochemical hallmarks of Zellweger syndrome. In all cases detailed studies were done in blood (very long chain fatty acids, bile acid intermediates, phytanic acid, pristanic acid, and pipercolic acid) and fibroblasts (de novo plasmalogen biosynthesis, very long chain fatty acids, oxidation of [1-¹⁴C]C26:0, pristanic acid, and phytanic acid, immunoblot analysis, dihydroxyacetonephosphate acyltransferase and alkyl-dihydroxyacetonephosphate synthase) (12). Informed consent was obtained from the parents and approval by legal authorities was obtained.

Preparation of human liver homogenates

Pieces of human liver from controls and Zellweger patients were stored at -70°C, thawed in a medium containing 250 mM mannitol, 5 mM MOPS-KOH, and 0.1 mM EDTA (pH 7.4), and homogenized. The whole homogenates were used for incubation studies. Protein concentrations were determined using an established method (13).

Differential centrifugation of human liver homogenates

Pieces of human liver tissue obtained from patients undergoing liver resection were immediately chilled in a medium containing 250 mM sucrose, 0.5 mM EDTA and 2 mM MOPS (final pH 7.4), finely minced and subjected to differential centrifugation exactly as described before (14). The marker enzymes glutamate dehydrogenase, catalase, and esterase were determined as described before (14, 15). Lactate dehydrogenase activity was assayed at 37°C by following the decrease in absorbance at 340 nm in a medium containing 50 mM potassium phosphate, 0.3 mM NADH, 0.1% (w/v) Triton X-100, 10 mM pyruvate, final pH 7.4, using a Cobas Fara II Centrifugal Analyzer (Hoffmann-La Roche, Basel, Switzerland).

Equilibrium density gradient centrifugation in a linear Nycodenz gradient was performed as described before (8).

Incubation conditions

Unless indicated otherwise, 2-hydroxyphytanic acid and 2-hydroxyphytanoyl-CoA metabolism was studied using a reaction medium (1 ml) containing, apart from human liver homogenate (1 mg protein), the following standard components: 150 mM KCl, 50 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES)-NaOH, 2 mM potassium phosphate, 10 μ M bovine serum albumin plus 0.02 mM substrate (2-hydroxyphytanic acid or 2-hydroxyphytanoyl-CoA). The final pH was 7.4. The following cofactors were added in different combinations: 3 mM MgCl₂, 2 mM ATP, 1 mM NAD⁺, 1 mM NADP⁺, 1 mM FAD, and 0.2 mM coenzyme A. Reactions were allowed to proceed for 80 min and terminated by adding 0.5 ml of 1 M HCl.

Quantification of 2-ketophytanic acid and pristanic acid was performed using gas chromatography-mass spectrometry (GC-MS) as described below.

Measurement of intermediates

Analysis of pristanic acid and 2,3-pristenic acid was performed as described before for pristanic acid in plasma samples (10), monitoring *m/z* 295 and 298 for 2,3-pristenic acid and its internal standard, respectively. For analysis of 2-ketophytanic acid, a 200- μ l aliquot of the reaction medium was taken and 0.05 nmol internal standard ([3-methyl-²H₃]-2-ketophytanic acid-ethyl ester) was added. After saponification and extraction with hexane, the samples were evaporated to dryness and converted into pentafluorobenzyl-esters in the same way as in the case of pristanic acid. After extraction to hexane and evaporation of the organic solvent, the sam-

TABLE 1. Formation of 2-ketophytanic acid and pristanic acid from 2-hydroxyphytanic acid or 2-hydroxyphytanoyl-CoA in human liver and its dependence on cofactors

Substrate	Cofactors	n	2-Ketophytanic Acid	Pristanic Acid
2-Hydroxyphytanic acid	none	3	5.3 ± 0.6	1.1 ± 0.3
2-Hydroxyphytanic acid	ATP, Mg ²⁺ , CoA	2	2.1–2.5	3.0–4.0
2-Hydroxyphytanic acid	NAD ⁺	4	3.9 ± 0.9	1.2 ± 0.2
2-Hydroxyphytanic acid	ATP, Mg ²⁺ , CoA, NAD ⁺	2	2.1–2.3	6.0–12.0
2-Hydroxyphytanoyl-CoA	none	3	0.0 ± 0.0	6.1 ± 1.3
2-Hydroxyphytanoyl-CoA	ATP, Mg ²⁺ , CoA	2	0.0–0.2	2.2–5.4
2-Hydroxyphytanoyl-CoA	NAD ⁺	3	0.3 ± 0.2	35.7 ± 2.5
2-Hydroxyphytanoyl-CoA	NADP ⁺	3	0.2 ± 0.1	33.1 ± 3.3
2-Hydroxyphytanoyl-CoA	FAD ⁺	2	0.0	3.9–9.7
2-Hydroxyphytanoyl-CoA	ATP, Mg ²⁺ , CoA, NAD ⁺	3	0.0 ± 0.0	23.1 ± 3.2

Intermediates are expressed as pmol/min per mg protein ± SD; n, number of experiments performed.

ples were acetylated with 100 μl pyridine and 100 μl acetic anhydride by heating for 90 min at 90°C. After evaporation, the residues were dissolved in 600 μl hexane and analyzed by GC–MS. The GC–MS conditions were similar to those described previously (8, 9).

RESULTS

2-Hydroxyphytanic acid metabolism in control liver homogenates

When control human liver homogenates were incubated with 2-hydroxyphytanic acid in the absence of exogenous cofactors, formation of 2-ketophytanic acid and pristanic acid was observed. When ATP, Mg²⁺, and coenzyme A were added to the reaction medium, the amount of pristanic acid that was formed increased significantly, whereas the 2-ketophytanic acid production diminished (Table 1). The most likely explanation for these findings is that the presence of these cofactors stimulates formation of 2-hydroxyphytanoyl-CoA from 2-hydroxyphytanic acid during incubation, after which 2-hydroxyphytanoyl-CoA is converted into pristanic acid (or pristanoyl-CoA). Addition of NAD⁺ (in the absence of ATP, Mg²⁺, and coenzyme A) did not influence formation of either 2-ketophytanic acid or pristanic acid. The combination of ATP, Mg²⁺, coenzyme A, and NAD⁺ (or NADP⁺) gave the highest yield of pristanic acid and the lowest of 2-ketophytanic acid, implying that NAD⁺ stimulated formation of pristanic acid from 2-hydroxyphytanoyl-CoA.

These observations, together with the recent finding of Mihalik, Rainville and Watkins (16) that 2-hydroxyphytanoyl-CoA is formed from phytanoyl-CoA, prompted us to use 2-hydroxyphytanoyl-CoA instead of 2-hydroxyphytanic acid for further studies.

2-Hydroxyphytanoyl-CoA metabolism in control liver homogenates

Using 2-hydroxyphytanoyl-CoA as the substrate, formation of 2-ketophytanic acid and pristanic acid was observed. The amount of 2-ketophytanic acid formed, however, was much lower than in incubations with 2-hydroxyphytanic acid as the substrate. On the other hand, the formation of pristanic acid was significantly higher and was not stimulated by added ATP, Mg²⁺, and coenzyme A (Table 1).

When NAD⁺ was added to the reaction medium, much higher rates of pristanic acid formation were found, suggesting that this cofactor is involved in the conversion of 2-hydroxyphytanoyl-CoA into pristanic acid as mentioned above. Surprisingly, NADP⁺ showed the same stimulatory effect as NAD⁺. FAD had no effect on the pristanic acid formation. Addition of ATP, Mg²⁺, and coenzyme A, in combination with NAD⁺, resulted in lower amounts of pristanic acid than in presence of NAD⁺ only. This was not due to lower activity of the decarboxylation, but was caused by further β-oxidation after activation of pristanic acid to pristanoyl-CoA, as is explained below (see section on the reaction product).

The formation of pristanic acid from 2-hydroxyphytanoyl-CoA, in the presence of NAD⁺, was linear for 80 min (Fig. 1). A linear relationship between the substrate concentration and formation of pristanic acid was found up to a concentration of 20 μM 2-hydroxyphytanoyl-CoA (results not shown). The optimal NAD⁺ concentration in the incubation medium was 1 mM (Fig. 1).

Identification of the reaction product

To investigate whether pristanic acid or pristanoyl-CoA was the reaction product of 2-hydroxyphytanoyl-CoA metabolism in human liver, we incubated a control human liver homogenate in the standard reaction me-

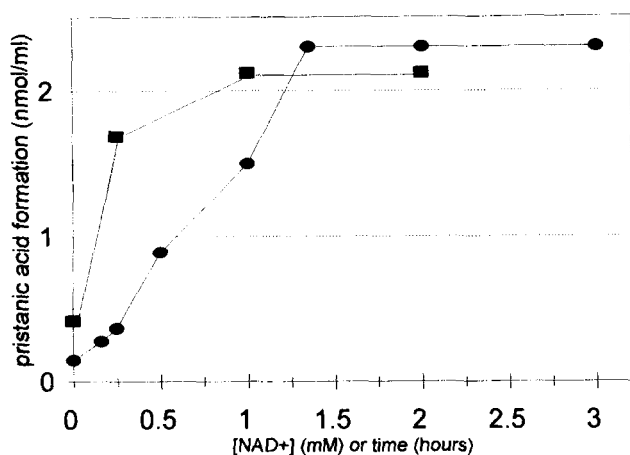


Fig. 1. Formation of pristanic acid from 2-hydroxyphytanoyl-CoA. Human liver homogenates were incubated with 2-hydroxyphytanoyl-CoA for different incubation periods in the presence of 1 mM NAD⁺ (●) or during 80 min in the presence of different NAD⁺ concentrations (■). Then the incubation medium was subjected to hydrolysis and pristanic acid concentrations were determined.

dium containing 2-hydroxyphytanoyl-CoA (0.05 mM) and NAD⁺ (1 mM). After 80 min, the reactions were terminated and the incubation medium was subjected to extraction with hexane, allowing extraction of pristanic acid but not pristanoyl-CoA. These experiments showed that pristanic acid was predominantly in the free carboxylic acid (unbound) form.

As this pristanic acid may have formed artefactually due to hydrolase activity, we sought additional evidence for formation of pristanic acid rather than pristanoyl-CoA. To this end we studied whether or not there was formation of 2,3-pristenic acid. The latter compound (in its CoA-ester form) is the expected metabolite if pristanoyl-CoA is the primary reaction product and not pristanic acid. This hypothesis was verified in the experiment of **Table 2** which showed formation of 2,3-pris-

tenic acid from pristanoyl-CoA but not from pristanic acid. In the incubations with 2-hydroxyphytanoyl-CoA as substrate, no formation of 2,3-pristenic acid was found. This strongly suggests that pristanic acid, and not pristanoyl-CoA, is the primary reaction product. Indeed, formation of 2,3-pristenic acid was only found when in addition to 2-hydroxyphytanoyl-CoA and NAD⁺, ATP, Mg²⁺, and coenzyme A were added to the incubation medium, allowing activation of pristanic acid to pristanoyl-CoA and further peroxisomal β -oxidation of this compound. The low ratio pristenic acid/pristanic acid (0.08) in incubations with 2-hydroxyphytanoyl-CoA in the presence of ATP, Mg²⁺, and coenzyme A implies that even though pristanic acid is further metabolized under these conditions, this is quantitatively a minor event. The amounts of ketophytanic acid and pristanic acid measured in the incubations therefore reflect most or all of the amounts actually formed.

Subcellular localization

In the experiment of **Fig. 2** we studied the subcellular localization of the NAD⁺-dependent formation of pristanic acid from 2-hydroxyphytanoyl-CoA. A human liver homogenate was subjected to differential centrifugation, yielding a nuclear, crude mitochondrial, microsomal, and cytosolic fraction. The results in **Fig. 2** show that formation of pristanic acid from 2-hydroxyphytanoyl-CoA has the same activity profile as esterase, suggesting a microsomal localization. To verify this finding, we performed an equilibrium density gradient centrifugation experiment using a linear Nycodenz gradient (for details, see ref. 8). Inclusion of Nycodenz in the incubation medium (0–5% w/v) did not influence the formation of pristanic acid from 2-hydroxyphytanoyl-CoA. **Figure 3** shows that pristanic acid formation showed a profile of activity similar to esterase, a marker enzyme for microsomes. The peroxisomal marker en-

TABLE 2. Formation of pristenic acid in relation to the amount of pristanic acid

Substrate	Cofactors	Pristenic Acid	Pristenic Acid/ Pristanic Acid
		nmol/ml	
Pristanic acid, 5 nmol	none	nd	—
Pristanic acid, 10 nmol	none	nd	—
Pristanic acid, 50 nmol	none	0.05	0.001
Pristanoyl-CoA, 5 nmol	none	0.84	0.17
Pristanoyl-CoA, 10 nmol	none	1.37	0.14
Pristanoyl-CoA, 50 nmol	none	6.81	0.14
2-Hydroxyphytanoyl-CoA, 50 nmol	NAD ⁺	0.003	0.001
2-Hydroxyphytanoyl-CoA, 50 nmol	NAD ⁺ , ATP, Mg ²⁺ , CoA	0.09	0.08

When incubating with pristanic acid (in the absence of cofactors), only a small amount of 2,3-pristenic acid is formed. When incubating with pristanoyl-CoA, the amount of 2,3-pristenic acid is significantly higher, as expressed by the ratio 2,3-pristenic acid/pristanic acid. In comparison, in the incubations in which 2-hydroxyphytanoyl-CoA is the substrate, the relative amount of 2,3-pristenic acid is low in the absence of ATP, Mg²⁺, and CoA and higher in the presence of these cofactors.

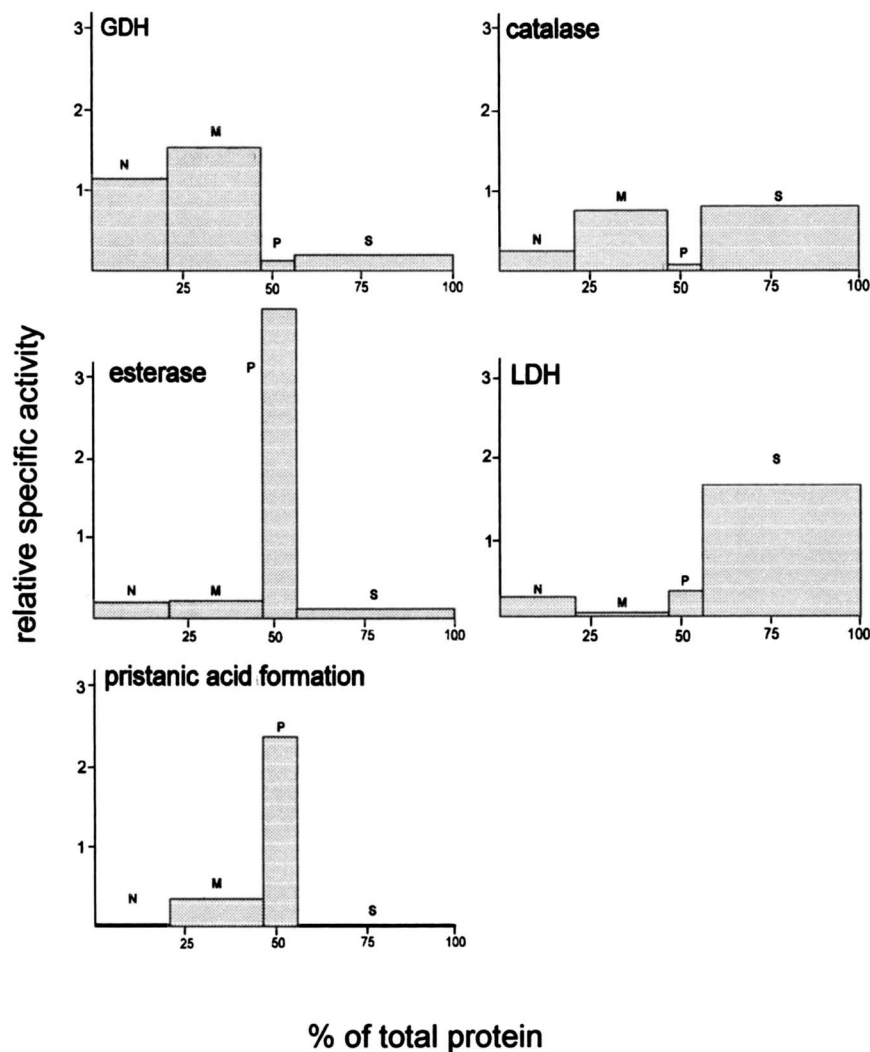


Fig. 2. Subcellular distribution of the formation of pristanic acid from 2-hydroxyphytanoyl-CoA in human liver. A liver homogenate was fractionated by differential centrifugation into nuclear (N), mitochondrial and peroxisomal (M), microsomal (P), and cytosolic (S) fractions and marker enzymes (LDH (lactate dehydrogenase): cytosolic marker enzyme, GDH (glutamate dehydrogenase): mitochondrial marker enzyme, catalase: peroxisomal marker enzyme, esterase: microsomal marker enzyme) and pristanic acid formation from 2-hydroxyphytanoyl-CoA were measured in each fraction. The relative activity in each fraction was calculated by dividing the actual activity (formation of pristanic acid per mg protein) in each fraction by the activity in the crude homogenate.

zyme, catalase, and the mitochondrial marker enzyme, glutamate dehydrogenase, displayed different activity patterns in the gradient fractions.

Activity measurements in Zellweger syndrome

In generalized peroxisomal disorders, phytanic acid accumulates in body fluids and tissues. To study the biochemical basis of this phenomenon, we incubated liver from a Zellweger patient with 2-hydroxyphytanoyl-CoA and 2-hydroxyphytanoyl-CoA. **Table 3** shows that the

conversion of 2-hydroxyphytanoyl-CoA into 2-ketophytanic acid was completely deficient in Zellweger syndrome, in accordance with previous results from our group (8). **Table 3** also shows that there was no formation of pristanic acid in incubations with 2-hydroxyphytanoyl-CoA.

When 2-hydroxyphytanoyl-CoA was used as substrate, there was no formation of 2-ketophytanic acid. In contrast, pristanic acid formation in the liver from a Zellweger patient was similar to the controls (**Table 3**).

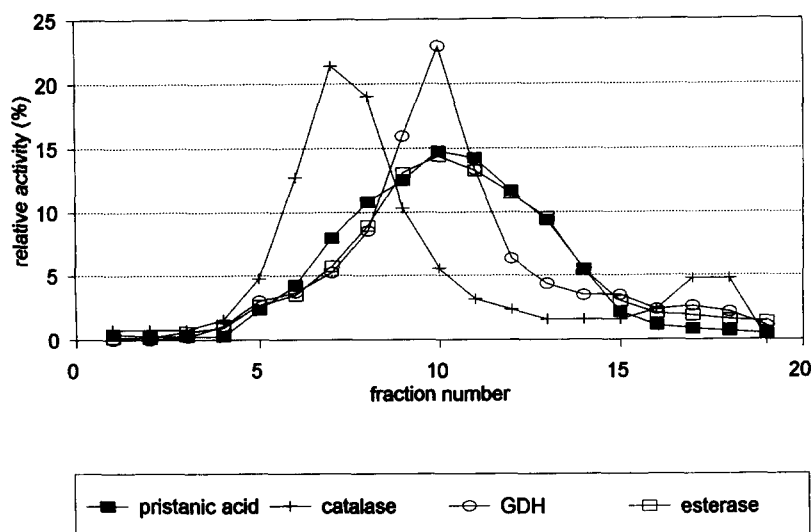


Fig. 3. Subcellular distribution of the formation of pristanic acid from 2-hydroxyphytanoyl-CoA in human liver. In all fractions, catalase (peroxisomal marker enzyme), esterase (microsomal marker enzyme), and glutamate dehydrogenase (mitochondrial marker enzyme) as well as pristanic acid formation was measured. The relative activity in each individual fraction was calculated by dividing the actual activity in each fraction by the sum total of activities measured in all fractions from the gradient.

DISCUSSION

In recent years, more insight into the α -oxidation pathway of phytanic acid has been gained. It was long thought that the breakdown of phytanic acid involved free carboxylic acid, and not the coenzyme A thioester (17, 18). However, recent data suggest that prior to oxidation, phytanic acid is activated to phytanoyl-CoA (4, 19). According to Pahan, Khan, and Singh (20, 21), this was only needed for transport across the peroxisomal membrane. From their experiments, in which intact peroxisomes were compared with permeabilized peroxisomes, they concluded that in the peroxisomal matrix, phytanoyl-CoA is hydrolyzed and that phytanic acid is the true substrate for α -oxidation. These findings are in striking contrast with the observations of Mihalik et al. (16). These authors have provided convincing evidence that phytanoyl-CoA is converted by a dioxygenase type of enzyme, phytanoyl-CoA hydroxylase, yielding 2-hydroxyphytanoyl-CoA (16). Their experiments, performed in rat liver, were extended by Jansen et al. (5–7) demonstrating that formation of 2-hydroxyphytanoyl-CoA also occurs in human liver and is deficient in liver from a Refsum patient, Zellweger patients, and

RCDP patients. The enzyme is localized in peroxisomes and uses 2-ketoglutarate as cosubstrate and Fe^{2+} and ascorbate as cofactors (5, 15).

Although the first step of phytanic acid α -oxidation, the conversion of phytanoyl-CoA into 2-hydroxyphytanoyl-CoA, is practically elucidated now, the remainder of the pathway is still incompletely understood. We reported earlier on the formation of 2-ketophytanic acid from 2-hydroxyphytanoyl-CoA in rat and human liver (8). The enzyme catalyzing this reaction was found to be localized in peroxisomes, at least in rat. The formation of ketophytanic acid was not enhanced by the addition of ATP, Mg^{2+} , and coenzyme A, showing that the enzymatic reaction was not dependent on activation of 2-hydroxyphytanoyl-CoA. In addition, neither NAD^+ nor NADP^+ was able to stimulate the reaction. The formation of 2-ketophytanic acid was completely deficient in liver obtained from a Zellweger patient.

It is generally accepted now that formate, and not CO_2 , is the primary reaction product of the decarboxylation of phytanic acid, as was shown both in vitro and in vivo (22, 23).

Experimental data on the decarboxylation of phytanic acid obtained so far can not yet be united to a

TABLE 3. Formation of 2-ketophytanic acid and pristanic acid from 2-hydroxyphytanoyl-CoA or 2-hydroxyphytanoyl-CoA in liver samples from controls and Zellweger patients

Substrate	Cofactors	Liver	n	2-Ketophytanic Acid	Pristanic Acid
2-Hydroxyphytanoyl-CoA	none	control	2	3.3–5.7	0.7–0.9
2-Hydroxyphytanoyl-CoA	none	Zellweger	2	0.0	0.0
2-Hydroxyphytanoyl-CoA	NAD^+	control	3	0.0–0.2	29.9–48.1
2-Hydroxyphytanoyl-CoA	NAD^+	Zellweger	2	0.0	30.0–40.0

Results are expressed as pmol/min per mg protein; n, number of different liver samples investigated.

clear metabolic pathway. Therefore, when it became clear that not 2-hydroxyphytanic acid but its coenzyme A thioester, 2-hydroxyphytanoyl-CoA, might be the true α -oxidation intermediate, we started to investigate conversion of this latter compound in human liver.

In the first experiments, we investigated the influence of additions of cofactors to incubations of control human liver with 2-hydroxyphytanic acid. These experiments were similar to those described before (8), with the exception that we now also monitored the formation of pristanic acid. This turned out to be of great value, as the formation of pristanic acid showed characteristics different from those in the formation of 2-ketophytanic acid. As described, highest concentrations of 2-ketophytanic acid were observed in the absence of added cofactors. However, in these incubations the rate of pristanic acid formation was very low. Formation of pristanic acid could be enhanced by addition of ATP, Mg^{2+} , and coenzyme A. Further stimulation occurred when NAD^+ or $NADP^+$ was added. However, concentrations of 2-ketophytanic acid diminished greatly. This suggests that after activation of 2-hydroxyphytanic acid to its coenzyme A thioester, $NAD(P)^+$ stimulated decarboxylation occurs, yielding pristanic acid. The formation of 2-ketophytanic acid, which is observed in the absence of cofactors for coenzyme A thioester formation, must probably be attributed to another pathway.

To further investigate this hypothesis, incubations with 2-hydroxyphytanoyl-CoA as substrate were performed. Only trace amounts of 2-ketophytanic acid were formed, whereas pristanic acid formation was higher than in the incubations with 2-hydroxyphytanic acid. Again, formation of pristanic acid was stimulated by NAD^+ or $NADP^+$.

Measurement of 2,3-pristenic acid in the different incubations with 2-hydroxyphytanoyl-CoA indicated that further oxidation of pristanic acid occurs only in the presence of ATP, Mg^{2+} , and coenzyme A. This is in line with earlier findings in similar incubations with pristanic acid, which showed absence of 2,3-pristenic acid formation when ATP, Mg^{2+} , and coenzyme A were not added (24).

The results obtained point to the existence of two pathways by which 2-hydroxyphytanic acid can be metabolized in vitro (Fig. 4). The first one, conversion of 2-hydroxyphytanic acid into 2-ketophytanic acid, is independent of all of the cofactors that were investigated. In principle, the pristanic acid found in incubations with 2-hydroxyphytanic acid can originate from 2-ketophytanic acid or could be formed after activation of 2-hydroxyphytanic acid to 2-hydroxyphytanoyl-CoA by small amounts of cofactors in the crude liver homogenates. Experimental results render the latter possibility unlikely. The observation that in Zellweger syndrome

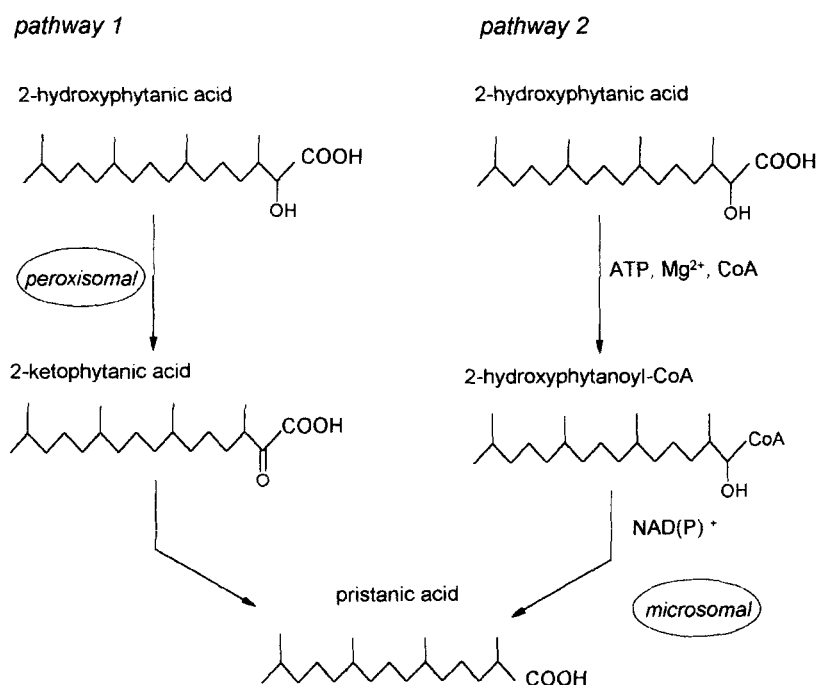


Fig. 4. Formation of pristanic acid from 2-hydroxyphytanic acid in human liver by the two different pathways found to occur in vitro.

no pristanic acid was found in incubations with 2-hydroxyphytanic acid strongly suggests that in the control livers pristanic acid originates from the 2-ketopristanic acid. As formation of pristanic acid from 2-hydroxyphytanoyl-CoA is not deficient in Zellweger syndrome, one would expect formation of pristanic acid despite absence of 2-ketophytanic acid formation, which is not the case. Whether or not 2-ketophytanic acid is enzymatically or spontaneously converted into pristanic acid cannot be concluded from our experiments. Enzymatic decarboxylation of 2-keto fatty acids has been reported to occur in brain tissue (25). However, we cannot rule out the possibility that only 2-ketophytanic acid is formed by liver, and the formation of pristanic acid occurs spontaneously. The second pathway, at least quantitatively more important than the first one, involves the formation of pristanic acid from 2-hydroxyphytanoyl-CoA. The product of this reaction is not pristanoyl-CoA, but pristanic acid. A determination of whether or not other intermediates are involved in this pathway will require further experiments.

In liver from a Zellweger patient, no degradation of 2-hydroxyphytanic acid could be observed. However, formation of pristanic acid from 2-hydroxyphytanoyl-CoA was similar to the controls. The only difference between the Zellweger and the controls when incubating with 2-hydroxyphytanoyl-CoA was the small amount of 2-ketophytanic acid found in the controls, which was absent in Zellweger livers. This suggests that 2-ketophytanic acid or 2-ketophytanoyl-CoA is not an intermediate in the conversion of 2-hydroxyphytanoyl-CoA to pristanic acid. The small amount of 2-ketophytanic acid that is found in the incubation of control liver with 2-hydroxyphytanoyl-CoA must originate from the conversion of 2-hydroxyphytanic acid, which is formed from 2-hydroxyphytanoyl-CoA by hydrolases present in liver.

The first pathway, formation of 2-ketophytanic acid from 2-hydroxyphytanic acid, is localized in peroxisomes, which can explain its deficiency in Zellweger syndrome. The microsomal pathway, formation of pristanic acid from 2-hydroxyphytanoyl-CoA, is normally active in Zellweger syndrome. In case this pathway is the one that functions in vivo, the accumulation of phytanic acid in this disorder must solely be ascribed to the deficiency of the first step, the conversion of phytanoyl-CoA into 2-hydroxyphytanoyl-CoA.

Most likely, as 2-hydroxyphytanoyl-CoA is formed from phytanoyl-CoA, pathway 2 as described in this paper is the one that is functionally active in vivo. Its microsomal localization implies a complicated physical organization of phytanic acid α -oxidation, involving both peroxisomes and microsomes. Future experiments are necessary to further investigate the existence of other

intermediates in the pathway and to establish the exact mechanism. ■■

Dr. Herman J. ten Brink is acknowledged for synthesis of the substrates used in this study. We thank Erwin Jansen, Ulbe Holwerda, and Rob Kok for their contribution to the measurements of the metabolites, and Simone Denis for her contribution in preparation of the subcellular fractions. We are indebted to Prof. Bwee Tien Poll-The for providing liver from a Zellweger patient.

Manuscript received 25 March 1997 and in revised form 5 June 1997.

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