Phytanic acid oxidation: normal activation and transport yet defective α-hydroxylation of phytanic acid in peroxisomes from Refsum disease and rhizomelic chondrodysplasia punctata

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Abstract In humans the oxidation of phytanic acid is a peroxisomal function. To understand the possible mechanisms for the pathognomic accumulation of phytanic acid in plasma and body fluids of Refsum disease (RD) and rhizomelic chondrodysplasia punctata (RCDP), we investigated activities of various steps (activation, transport, and oxidation) in the metabolism of phytanic acid in peroxisomes isolated from cultured skin fibroblasts from control, RD, and RCDP subjects. Activation of phytanic acid was normal in peroxisomes from both RD and RCDP. Transport of phytanic acid or phytanoyl-CoA in the absence or presence of fatty acid activating cofactors (ATP, $MgCl_2$, and CoASH) into peroxisomes isolated from RD and RCDP skin fibroblasts was also similar to that of peroxisomes from control fibroblasts. Defective oxidation of [(2,3)-^{3}H]- or [1-^{14}C]phytanic acid, or [1-^{14}C]phytanoyl-CoA (substrate for the first step of α-oxidation) but normal oxidation of [1-^{14}C]α-hydroxyphytanic acid (substrate for the second step of the α-oxidation pathway) in peroxisomes from RD clearly demonstrates that excessive accumulation of phytanic acid in plasma and body fluids of RD is due to the deficiency of phytanic acid α-hydroxylase in peroxisomes. However, in RCDP peroxisomes, in addition to deficient oxidation of [1-^{14}C]phytanic acid or phytanoyl-CoA or [(2,3)-^{3}H]phytanic acid, the oxidation of [1-^{14}C]α-hydroxyphytanic acid was also deficient, indicating that in RCDP the activities both of α-hydroxylation of phytanic acid and decarboxylation of α-hydroxyphytanic acid are deficient. These observations indicate that peroxisomal membrane functions (phytanic acid activation and transport) in phytanic acid metabolism are normal in both RD and RCDP. The defect in RD is in the α-hydroxylation of phytanic acid; whereas in RCDP both α-hydroxylation of phytanic acid as well as decarboxylation of α-hydroxyphytanic acid are deficient.—**Pahan, K., M. Khan, and I. Singh.** Phytanic acid oxidation: normal activation and transport yet defective α-hydroxylation of phytanic acid in peroxisomes from Refsum disease and rhizomelic chondrodysplasia punctata. *J. Lipid Res.* 1996. **37:** 1137–1143.

Supplementary key words Refsum disease • α-oxidation

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), a saturated branched-chain fatty acid, is present in trace amounts in healthy human beings. Phytanic acid originates mainly from dietary sources and little, if any, is synthesized in mammals (1). The daily dietary intake is estimated to be 50–100 mg in humans. Due to a defect in its catabolism it accumulates in excessive amounts in tissues and body fluids of patients with classical Refsum disease (RD), rhizomelic chondrodysplasia punctata (RCDP), and in patients with defects in the biogenesis of peroxisomes (e.g., Zellweger syndrome, infantile Refsum disease, hyperpipecolic acidemia, and neonatal adrenoleukodystrophy) (1, 2).

Due to the presence of a beta methyl group, the phytanic acid is first α-oxidized to its lower homologue, pristanic acid, which is then further catabolized by β-oxidation. Recently, we have demonstrated that in humans, α-oxidation of phytanic acid to pristanic acid takes place in peroxisomes (3–7) but peroxisomes from RD and RCDP are deficient in this activity (4, 5). Phytanoyl-CoA ligase is present in the peroxisomal membrane whereas the enzyme system involved in the metabolic steps for α-oxidation is present in the peroxisomal matrix (6). The conversion of phytanic acid to phytanoyl-CoA, a prerequisite for its transport into peroxisomes, takes place on the cytoplasmic surface of peroxisomal membrane, and phytanoyl-CoA is hydrolyzed to phytanic acid prior to its α-oxidation to pristanic acid in the matrix of peroxisomes (6, 7). The α-oxidation of phytanic acid in the peroxisomal matrix involves at least three steps: *1)* α-hydroxylation of phytanic acid to α-hydroxyphytanic acid; *2)* conversion of α-hydroxyphytanic acid to α-ketophytanic acid; and *3)* decarboxylation of α-ketophytanic

Abbreviations: RCDP, ehizomelic chondrodysplasia punctata; RD, Refsum disease; AMPCPOP, α,β-methyleneadenosine 5′ triphosphate.

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acid to pristanic acid (**Fig. 1**). Herndon and associates (8) have previously reported that monolayers of cultured skin fibroblasts from RD patients are deficient in the oxidation of phytanic acid but capable of oxidizing both α-hydroxyphytanic acid and pristanic acid. This suggests that the defect in the α-oxidation of phytanic acid in RD is in the α-hydroxylation step. On the other hand, the specific biochemical step that is deficient in the α-oxidation of phytanic acid in RCDP is not yet known. As peroxisomes have now been identified to be responsible for α-oxidation of phytanic acid, and both activation and transport of phytanic acid occur prior to the formation of α-hydroxyphytanic acid, it is of particular interest to determine whether peroxisomes from both RD and RCDP have normal activities for the activation and transport of phytanic acid or for the formation and oxidation of α-hydroxyphytanic acid in purified peroxisomes.

In a systematic effort to elucidate the specific defect in the pathway of phytanic acid α-oxidation in both RD and RCDP, we examined the rates of activation and transport of phytanic acid, and for the oxidation of both phytanic acid and α-hydroxyphytanic acid in peroxisomes isolated from cultured skin fibroblasts from RD, RCDP, and control subjects. In this article, we report that peroxisomes from both RD and RCDP have normal activities for the activation and transport of phytanic acid, and that peroxisomes from RD are deficient in the α-hydroxylation of phytanic acid while those from RCDP are deficient in α-hydroxylation of phytanic acid as well as in the decarboxylation of the α-hydroxyphytanic acid to pristanic acid.

MATERIALS AND METHODS

Nycodenz was obtained from Accurate Chemical and Scientific Corp., Westbury, NY. ATP and CoASH were purchased from P-L Biochemicals, Milwaukee, WI. Cytochrome c, α-cyclodextrin, FAD, and α, β-methyleneadenosine 5′ triphosphate (AMPCPOP) were purchased from Sigma Chemical Co., St. Louis, MO. [1-^{14}C]phytanic acid (55 mCi/mmol) was purchased from Amersham International, Arlington Heights, IL. [1-^{14}C]phytanoyl-CoA was synthesized as described by Akanuma and Kishimoto (9). [(2,3)-^{3}H]phytanic acid was synthesized from [(2,3)-^{3}H]dihydrophytol as described by Zenger-Hain, Craft, and Riggs (10). [1-^{14}C]α-hydroxyphytanic acid was synthesized from [1-^{14}C]phytanic acid as described by Skjeldal and Stokke (11). Briefly, [1-^{14}C]phytanic acid was converted to [1-^{14}C]phytanoyl chloride by treatment with oxalyl chloride that was brominated to [1-^{14}C]α-bromophytanoyl chloride. After removing excess bromine and solvent, [1-^{14}C]α-bromophytanoyl chloride was hydrolyzed to [1-^{14}C]α-hydroxyphytanic acid by KOH and ethanol and further purified by TLC.

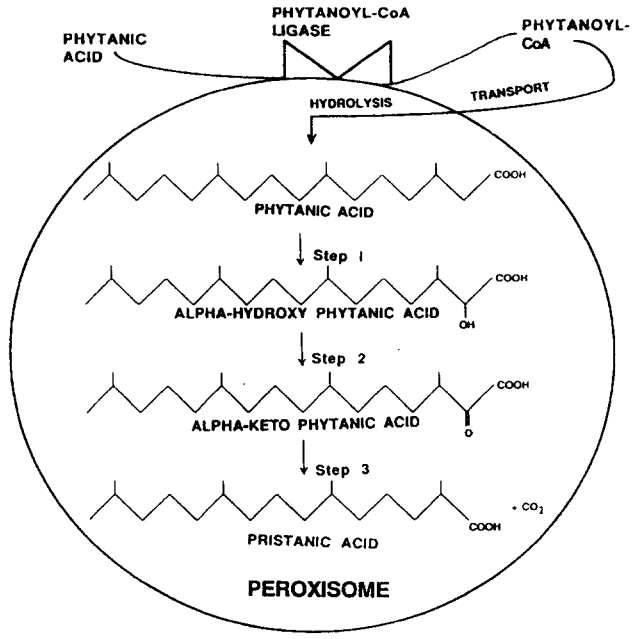

Fig. 1. Schematic drawing of pathway of phytanic acid α-oxidation.

Isolation of peroxisomes from cultured skin fibroblasts

Fibroblast cell lines from control, Refsum, and RCDP subjects were cultured and fractionated as described previously (12). Briefly, the cellular homogenates were centrifuged at 500 *g* for 5 min to obtain the postnuclear (PN) fraction that was further fractionated by isopycnic equilibrium centrifugation in continuous Nycodenz gradients. Tubes (39 ml) for a Beckman JV-20 rotor were layered with 4 ml of 35% (w/v) Nycodenz and 28 ml of a continuous gradient consisting of 0–30% (w/v) Nycodenz in homogenization medium (0.25 M sucrose, 1 mM EDTA, 0.5 μg/ml leupeptin, 0.5 μg/ml antipain, 0.7 μg/ml pepstatin, 0.2 mM phenylmethylsulfonyl fluoride, and 0.1% ethanol in 3 mM imidazole buffer, pH 7.4). The PN-fraction was applied on top of the Nycodenz layers and the tubes were centrifuged at 33,747 *g* for 1 h. The gradient fractions were analyzed for the following subcellular markers: cytochrome c oxidase for mitochondria (13), NADPH cytochrome c reductase for microsomes (14), and catalase for peroxisomes (15). Protein concentration was determined by the procedure of Bradford (16). The three fractions with the highest catalase activity from the gradient were pooled and dialyzed against the homogenization buffer for 1 h to lower the concentration of Nycodenz (4).

Activation and oxidation of fatty acids

The activity for phytanoyl-CoA ligase was measured as described previously (5–7). Briefly, the reaction mixture in 0.25 ml contained 12 μM [1-^{14}C]phytanic acid, 50 mM KCl, 5 mM $MgCl_2$, 50 μM CoASH, and 30 mM MOPS-HCl buffer, pH 7.8. The reaction was stopped by Dole's reagent and the radioactivity in the aqueous layer was measured as an index of amount of phytanoyl-CoA.

The α-oxidation of [1-^{14}C]phytanic acid and [1-^{14}C]α-hydroxyphytanic acid was measured as liberated $^{14}CO_2$ as described previously (3, 4). Briefly, the reaction volume of 0.25 ml contained 12 μM fatty acid, 30 mM KCl, 5 mM $MgCl_2$, 50 μM CoASH, 10 mM ATP, 0.25 mM NADPH, 0.17 mM FAD, and 20 mM MOPS-HCl buffer, pH 7.8. The reaction was started by the addition of 10–50 μg of protein and was stopped with 200 μl of 4 M H_2SO_4. The $^{14}CO_2$ was measured as an index of α-oxidation of phytanic acid. Assay for the oxidation of [(2,3)-^{3}H]phytanic acid was performed essentially as described by Zenger-Hain et al. (10). In all the cases, fatty acids were solubilized with α-cyclodextrin as follows. Fatty acids (15×10^6 dpm) were first dried in a tube under nitrogen and then resuspended in 2 ml of 0.25 M sucrose buffer (20 mg α-cyclodextrin/ml) by sonication for 30 min.

Transport of phytanic acid into peroxisomes

Peroxisomal fractions were incubated under isotonic conditions (homogenization buffer) with [1-^{14}C]phytanic acid or [1-^{14}C]phytanoyl-CoA solubilized with α-cyclodextrin in the presence or absence of CoASH and ATP or their analogs as described earlier (7). After the incubation period, peroxisomes were separated from the incubation medium by centrifugation through an organic layer of brominated hydrocarbons (17, 18). Briefly, microtubes (1.0 ml) contained 50 μl of 0.396 M sucrose buffer containing 1 mM imidazole and 3 mM disodium EDTA, pH 7.4, as cushion; an organic layer (400 μl) consisted of a mixture of bromododecane and bromodecane (7:4, v/v), and an upper layer (500 μl) consisted of 0.25 M sucrose, 30 mM MOPS buffer, pH 7.8. The concentrations of substrates and cofactors used were 12 μM for [1-^{14}C]phytanic acid or [1-^{14}C]phytanoyl-CoA, 50 μM CoASH, 5 mM $MgCl_2$, 10 mM ATP, or 2 mM ATP analog (AMPCPOP). Incubation was started with the addition of 5–25 μg of peroxisomal protein. After 5 min incubation at 25°C, the reaction mixture was transferred to the top of microtubes containing the organic layer and centrifuged in a Beckman TL-100 ultracentrifuge (TLA-100.2 rotor) at 9130 *g* for 8 min with low acceleration and deceleration. The upper aqueous and organic layers were removed. The radioactivity in the bottom layer containing the sedimented peroxisomes was measured. Catalase activity and latency of catalase were measured in the upper and bottom aqueous layers. Appropriate blanks with disrupted peroxisomes (sonicated) and another set without peroxisomes were included in these studies. The rates of transport for phytanic acid and phytanoyl-CoA using disrupted (by sonication) peroxisomes (blanks) were 3.6, and 3.45%, respectively, of the rates of these fatty acids transported into intact peroxisomes.

RESULTS

Activation of phytanic acid in peroxisomes from control, RD, and RCDP cultured skin fibroblasts

Activation of phytanic acid to phytanoyl-CoA is the initial and obligatory step in the α-oxidation of phytanic acid in intact peroxisomes in the cell. Therefore, to identify the specific defect in the pathway of phytanic acid α-oxidation in RD and RCDP, we first examined activation of phytanic acid in peroxisomes isolated from control, RD, and RCDP cultured skin fibroblasts. The peroxisomes isolated from cultured skin fibroblasts from control, RD, and RCDP were of approximately 90% percent purity with only relatively minor contamination by mitochondria (5.5–6.2%) and microsomes (3.2–3.5%) as protein. Percent purity of the peroxisomal fractions was calculated according to the method of Fujiki et al. (19). As seen in **Table 1,** the activation of phytanic acid (the activity of phytanoyl-CoA ligase) in peroxisomes from both RD and RCDP was similar to that of control suggesting that the first step of phytanic acid catabolism, the conversion of phytanic acid to phytanoyl-CoA, is normal in these two diseases. In agreement with previous observations (5, 20), the activity of phytanoyl-CoA ligase in peroxisomes from control, RD, and RCDP fibroblasts was 5- to 8-fold higher than in mitochondria or microsomes (Table 1).

TABLE 1. Activation of phytanic acid in different subcellular organelles isolated from cultured skin fibroblasts of control, RD, and RCDP

	Phytanoyl-CoA Ligase			
	Homogenate	Mitochondria	Microsomes	Peroxisomes
	nmol/h/mg protein			
Control	4.18 ± 0.65	3.82 ± 0.42	5.38 ± 0.84	26.5 ± 3.9
RD	3.97 ± 0.55	3.76 ± 0.35	5.12 ± 1.21	22.9 ± 4.2
RCDP	4.28 ± 0.82	4.15 ± 0.74	5.02 ± 0.48	29.2 ± 4.6

Activity for phytanoyl-CoA ligase was measured as described in the text. Results are expressed as mean ± SD of three different experiments.

TABLE 2. Transport of phytanic acid and phytanoyl-CoA into peroxisomes isolated from cultured skin fibroblasts of control, RD, and RCDP

Substrates	Rate of Transport			
	+ATP/+CoASH/+$MgCl_2$	-ATP/+AMPCPOP	-ATP/-CoASH/-$MgCl_2$	-CoASH/+desulfo-CoA Agarose
	nmol/h/mg protein			
Control				
Phytanic acid	6.57 ± 1.20	0.63 ± 0.14	0.57 ± 0.15	0.52 ± 0.09
Phytanoyl-CoA	6.64 ± 0.85	6.72 ± 1.32	5.65 ± 0.90	6.61 ± 0.65
RD				
Phytanic acid	7.22 ± 1.66	0.69 ± 1.10	0.72 ± 0.12	0.65 ± 0.13
Phytanoyl-CoA	7.37 ± 0.9	6.96 ± 1.25	7.18 ± 1.20	7.26 ± 0.98
RCDP				
Phytanic acid	5.21 ± 0.82	0.36 ± 0.06	0.42 ± 0.05	0.48 ± 0.11
Phytanoyl-CoA	5.47 ± 0.62	5.66 ± 1.10	5.42 ± 0.75	5.61 ± 0.45

Transport of phytanic acid and phytanoyl-CoA into peroxisomes in the presence (+) or absence (-) of different cofactors was measured as described in the text. The results are expressed as mean ± SD of three separate experiments.

Transport of phytanic acid and phytanoyl-CoA into peroxisomes of control, RD, and RCDP

Previous studies have shown that in human liver peroxisomes phytanic acid is transported as its thioester derivative, phytanoyl-CoA, and not as a free fatty acid (7). The relative rates of transport of phytanic acid/phytanoyl-CoA in the presence or absence of fatty acid-activating conditions are summarized in **Table 2.** The integrity (76–84%) of peroxisomes from control, RD, and RCDP was similar and was also not affected by the various experimental conditions (e.g., dialysis, centrifugation, and effects of phytanic acid and phytanoyl-CoA) used to study the transport of phytanic acid (data not shown). The transport of phytanic acid was linear between 5 and 25 μg of peroxisomal protein (**Fig. 2**). Therefore, for the following studies, the transport of fatty acids was examined with 5 μg of peroxisomal protein for 5 min at 25°C. The rates of transport of phytanoyl-CoA (6.64 ± 0.85 nmol/h per mg protein) and phytanic acid in the fatty acid-activating medium (6.57 ± 1.20 nmol/h per mg protein) into control peroxisomes were nearly similar. However, the rate of transport of phytanic acid (in the absence of CoASH, ATP, and $MgCl_2$) was only 8% as compared with that of phytanoyl-CoA. The transport of phytanic acid was dependent on the presence of CoASH, ATP, and $MgCl_2$ in the assay medium (complete medium for activation of fatty acids), whereas the transport of phytanoyl-CoA appeared to be independent of these cofactors (Fig. 2 and Table 2). Although phytanic acid requires activation to phytanoyl-CoA prior to its transport, the transport of phytanoyl-CoA in the absence of ATP and CoASH suggests that the translocation of phytanoyl-CoA across the membrane is not energy (ATP)-dependent (Table 2). These conclusions are also supported by the findings that the transport of phytanic acid is inhibited by the substitution of ATP and/or CoASH with their respective analogs (AMPCPOP or desulfoCoA-agarose) which do not support the activation of phytanic acid to

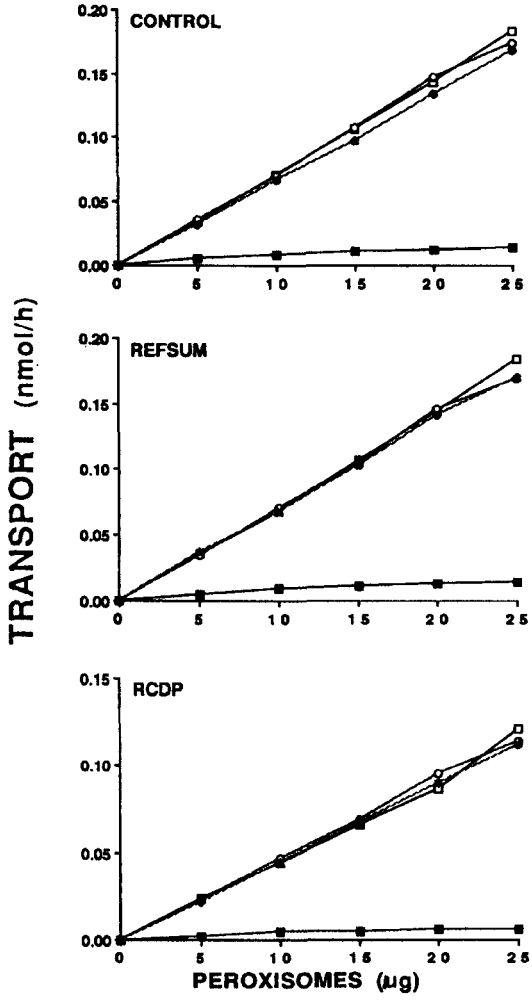

Fig. 2. Transport of phytanic acid and phytanoyl-CoA into peroxisomes isolated from control, RD, and RCDP cultured skin fibroblasts. The transport of phytanic acid (□, in the presence and ■, in the absence of fatty acid activating conditions) and phytanoyl-CoA (○, in the presence and ●, in the absence of fatty acid activating conditions) was studied in purified peroxisomes as described in the text. The results are the average of two independent determinations done in duplicate.

TABLE 3. Oxidation of α-hydroxyphytanic acid in different subcellular organelles isolated from cultured skin fibroblasts of control, RD, and RCDP

	Rate of Oxidation			
	Homogenate	Mitochondria	Microsomes	Peroxisomes
	pmol/h/mg protein			
Control	2.21 ± 0.32	1.68 ± 0.28	0.36 ± 0.06	26.4 ± 3.8
RD	2.08 ± 0.35	1.89 ± 0.34	0.22 ± 0.04	23.6 ± 3.5
RCDP	0.32 ± 0.11	0.41 ± 0.12	0.12 ± 0.02	3.7 ± 0.65

Oxidation of α-hydroxyphytanic acid was carried out as described in the text. Results are expressed as mean ± SD of three different experiments.

phytanoyl-CoA. On the other hand, AMPCPOP and desulfoCoA-agarose had no effect on the transport of phytanoyl-CoA (Table 2). These results demonstrate that similar to liver peroxisomes (7), the phytanic acid needs to be converted to phytanoyl-CoA by phytanoyl-CoA ligase prior to its transport into peroxisomes isolated from human skin fibroblasts. However, no phytanic acid oxidation activity is observed in the absence of fatty acid-activating conditions in the intact peroxisomes even though true substrate for α-oxidation enzyme system is free phytanic acid. The lack of this activity suggests that the fatty acid transported or diffused into peroxisomes under these conditions is not accessible to the α-oxidation system, present in the matrix of peroxisomes. Moreover, there was no difference in the rates of transport of phytanic acid or phytanoyl-CoA into peroxisomes from control, RD and RCDP (Fig. 2 and Table 2) suggesting that the transport of phytanic acid into peroxisomes from RD and RCDP is normal.

Subcellular localization of α-hydroxyphytanic acid oxidation in cultured skin fibroblasts from control, RD, and RCDP

α-Hydroxyphytanic acid was proposed as an intermediate in the α-oxidation of phytanic acid to pristanic acid in studies on monolayers of cultured skin fibroblasts (8, 21). The relative rates of oxidation of α-hydroxyphytanic acid by different subcellular organelles are summarized in **Table 3.** The rates of decarboxylation of [1-^{14}C]α-hydroxyphytanic acid in peroxisomes isolated from both control and RD were 12- to 16-times higher than those in mitochondria, and 73- to 107-times higher than those in microsomes, suggesting that oxidative decarboxylation of α-hydroxyphytanic acid occurs primarily in peroxisomes. The rates of oxidation of α-hydroxyphytanic acid in microsomes, mitochondria, and peroxisomal fractions from control fibroblasts were similar to the respective organelles from RD fibroblasts. However, the rate of oxidation of α-hydroxyphytanic acid was lower in RCDP than controls. The rate of oxidation of α-hydroxyphytanic acid in peroxisomes isolated from RCDP was only 14–16% of rates observed in peroxisomes from either control or RD (Table 3).

Oxidation of phytanic acid, phytanoyl-CoA, and α-hydroxyphytanic acid in peroxisomes

The rates of oxidation of phytanic acid, phytanoyl-CoA, and α-hydroxyphytanic acid in peroxisomes isolated from cultured skin fibroblasts of control, RD, and RCDP are summarized in **Table 4.** Consistent with the previous observations (4, 5), the deficient α-oxidation of both [1-^{14}C]phytanic acid and [1-^{14}C]phytanoyl-CoA in isolated peroxisomes again suggests that oxidation of phytanic acid is defective in RD or RCDP. It has been demonstrated that the tritium release from [(2,3)-^{3}H]phytanic acid is deficient in skin fibroblasts of RD, RCDP, and Zellweger subjects and that this assay can be used as a measure of phytanic acid α-oxidation (10). The measurement of tritium released from [(2,3)-^{3}H]phytanic acid does not provide a selective measure of α-oxidation, but the removal of the label as $[^{3}H]_2O$ from α-carbon of [(2,3)-^{3}H]phytanic acid should theoretically be considered as the index of α-oxidation and more specifically as the α-hydroxylation of phytanic acid (10). The deficient oxidation of [(2,3)-^{3}H]phytanic acid in peroxisomes of RD and RCDP indicate that both of these disorders have a defect in α-hydroxylation of phytanic acid. Defective oxidation of both [1-^{14}C]phytanic acid and [(2,3)-^{3}H]phytanic acid (substrates for the first step of α-oxidation) but normal oxidation of [1-^{14}C]α-hydroxyphytanic acid (substrate for the second step of α-oxidation) in peroxisomes of RD clearly demonstrate a defect in the phytanic acid

TABLE 4. Oxidation of different derivatives of phytanic acid in peroxisomes isolated from cultured skin fibroblasts of control, RD, and RCDP

	Rate of Oxidation			
	[1-^{14}C]Phytanic Acid	[1-^{14}C]Phytanoyl-CoA	[(2,3)^{3}H]Phytanic Acid	[1-^{14}C]α-Hydroxyphytanic Acid
	pmol/h/mg protein			
Control	38.6 ± 4.60	35.2 ± 5.20	28.3 ± 4.90	26.4 ± 3.80
RD	0.00	0.00	0.86 ± 0.15	23.6 ± 3.20
RCDP	0.00	0.00	1.32 ± 0.28	3.70 ± 0.65

Oxidation of phytanic acid was measured as described in the text. Results are expressed as mean ± SD of three experiments.

α-hydroxylation activity in peroxisomes (Fig. 1 and Table 4). Deficiencies in the α-oxidation of substrates for the first step ([1-^{14}C]phytanic acid, [(2,3)-^{3}H]phytanic acid) and for the second step ([1-^{14}C]α-hydroxyphytanic acid) in the catabolic pathways of phytanic acid in peroxisomes from RCDP demonstrate defects both in the formation of α-hydroxyphytanic acid as well as its oxidative decarboxylation to pristanic acid (Fig. 1 and Table 4).

DISCUSSION

The rate of oxidation of phytanic acid at a level of 3–5% of the respective controls in two RD patients and cultured skin fibroblasts from RD patients suggests that the degree of the defect in the phytanic acid α-oxidation in RD patients and cultured skin fibroblasts from RD patients is similar (1, 8, 21). The deficient oxidation of phytanic acid as compared to normal oxidation of α-hydroxyphytanic acid in cultured skin fibroblast monolayers of RD patients suggests a defect in the conversion of phytanic acid to α-hydroxyphytanic acid (1, 8); however, the subcellular organelle responsible for this function was not identified. Previously we reported that in human liver as well as in cultured skin fibroblasts the oxidation of phytanic acid to pristanic acid is a peroxisomal function (3, 4) and that this function is deficient in RD and RCDP (Tables 3 and 4). This defect in the peroxisomal oxidation may be responsible for the pathognomonic accumulation of phytanic acid in tissues from RD and RCDP (1, 2).

Identification of the specific step(s) responsible for the α-oxidation of phytanic acid is important for understanding the pathophysiology of disease process in disorders in which there is accumulation of excessive amounts of phytanic acid. The oxidation of phytanic acid to pristanic acid involves a number of steps as shown in Fig. 1. The activation of phytanic acid to phytanoyl-CoA by phytanoyl-CoA ligase, localized in the peroxisomal membrane, is prerequisite for its oxidation in intact peroxisomes (6). The active site of phytanoyl-CoA ligase is on the cytoplasmic surface of peroxisomes and phytanic acid is transported into peroxisomes as phytanoyl-CoA and then hydrolyzed to free phytanic acid prior to its oxidation in the peroxisomal matrix (6, 7). The similar rates of activation of phytanic acid to phytanoyl-CoA in peroxisomes from both RD and RCDP skin fibroblasts indicate that peroxisomes from RD and RCDP have normal activity of phytanoyl-CoA ligase. Studies on phytanic acid/phytanoyl-CoA transport, in the presence or in the absence of fatty acid-activating conditions, in peroxisomes isolated from RD, RCDP, and control fibroblasts have clearly demonstrated that translocation of phytanic acid through the peroxisomal membrane requires the activation of phytanic acid to phytanoyl-CoA and that the transport of phytanoyl-CoA into peroxisomes is normal in RD and RCDP. The normal activation and transport of phytanic acid in peroxisomes in RD and RCDP suggest that the functions of the peroxisomal limiting membrane in the oxidation of phytanic acid in these two disorders are normal (Fig. 2, Tables 1 and 2). This suggests that the defective α-oxidation of phytanic acid in RD and RCDP may be due to an abnormality in subsequent steps that take place in the peroxisomal matrix (Fig. 1).

The α-oxidation of phytanic acid to pristanic acid in the peroxisomal matrix involves at least three steps (Fig. 1). The conversion of phytanic acid to α-hydroxyphytanic acid in peroxisomes is carried out by a cytochrome P450-containing monooxygenase (22), and the oxidation of α-hydroxyphytanic acid to α-ketophytanic acid is carried out by an H_2O_2-producing α-hydroxyphytanic acid oxidase (23). Here our studies on the subcellular localization of α-hydroxyphytanic acid oxidase in cultured skin fibroblasts provide evidence that this activity is primarily localized in peroxisomes. We believe that α-ketophytanic acid is an unstable compound, and is therefore spontaneously converted to pristanic acid. The normal oxidation of α-hydroxyphytanic acid as compared to the deficient oxidation of [(2,3)-^{3}H]phytanic acid in peroxisomes from RD fibroblasts demonstrated that the defect in the α-oxidation of phytanic acid in RD lies in the α-hydroxylation of phytanic acid. On the other hand, the defective oxidation of both [(2,3)-^{3}H]phytanic acid and α-hydroxyphytanic acid in peroxisomes from RCDP indicates that in RCDP both the formation of α-hydroxyphytanic acid as well as the oxidation of α-hydroxyphytanic acid is impaired.

Peroxisomal proteins are synthesized on free polysomes and post-translationally imported into preexisting peroxisomes, and new peroxisomes are made by fission of existing peroxisomes (24). So far two peroxisomal targeting signals (PTS), PTS1 and PTS2, for matrix proteins have been identified (24). The majority of mature matrix proteins contain the PTS1 motif, Ser-Lys-Leu or its variant, at the C-terminal end. The PTS2 motif is localized at the N-terminal end of the precursor protein and only one such protein, 3-ketoacyl-CoA thiolase, has been identified in mammals (24). Recently, Slawecki et al. (25) have reported that RCDP is caused by a defect in import of PTS2-containing proteins. The normal activity of PTS-2 signal in RD as compared to a defect in RCDP indicate that the import of α-hydroxyphytanic acid oxidase into peroxisomes may possibly be mediated via the PTS-2 motif.

In summary, the studies reported in this article dem-

onstrate that in humans the oxidation of α-hydroxy phytanic acid is a peroxisomal function. The similar rates of activation and transport of phytanic acid in peroxisomes from RD, RCDP, and control subjects indicate that the functions of peroxisomal limiting membrane in the oxidation of phytanic acid are normal. The defective oxidation of [(2,3)-^{3}H]phytanic acid, but normal oxidation of α-hydroxyphytanic acid, clearly delineates RD as a peroxisomal disorder with a single enzyme defect in the α-hydroxylation of phytanic acid. The impaired oxidation of both [(2,3)-^{3}H]phytanic acid and α-hydroxyphytanic acid in RCDP suggests a defect in both of these metabolic steps.

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