Substrate Specificities of Rat Liver Peroxisomal Acyl-CoA Oxidases: Palmitoyl-CoA Oxidase (Inducible Acyl-CoA Oxidase), Pristanoyl-CoA Oxidase (Non-inducible Acyl-CoA Oxidase), and Trihydroxyprostanoyl-CoA Oxidase*

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Rat liver peroxisomes contain three acyl-CoA oxidases: palmitoyl-CoA oxidase, pristanoyl-CoA oxidase, and trihydroxyprostanoyl-CoA oxidase. The three oxidases were separated by anion-exchange chromatography of a partially purified oxidation preparation, and the column eluate was analyzed for oxidation activity with different acyl-CoAs.

Short chain mono (hexanoyl-) and dicarboxylyl (glutaryl-) CoAs and prostaglandin E2-CoA were oxidized exclusively by palmitoyl-CoA oxidase. Long chain mono (palmitoyl-) and dicarboxylyl (hexadecanoyl-) CoAs were oxidized by palmitoyl-CoA oxidase and pristanoyl-CoA oxidase, the former enzyme catalyzing about 70% of the total eluate activity. The very long chain lignoceryl-CoA was also oxidized by palmitoyl-CoA oxidase and pristanoyl-CoA oxidase, the latter enzyme catalyzing about 65% of the total eluate activity. Long chain 2-methyl branched acyl-CoAs (2-methylpalmitoyl-CoA and pristanoyl-CoA) were oxidized for about 90% by pristanoyl-CoA oxidase, the remaining activity being catalyzed by trihydroxycoprostanoyl-CoA oxidase. The short chain 2-methylhexanoyl-CoA was oxidized by trihydroxycoprostanoyl-CoA oxidase and pristanoyl-CoA oxidase (~60 and 40%, respectively, of the total eluate activity). Trihydroxycoprostanoyl-CoA was oxidized exclusively by trihydroxyprostanoyl-CoA oxidase. No oxidase activity was found with isovaleryl-CoA and isobutyryl-CoA.

Substrate dependences of palmitoyl-CoA oxidase and pristanoyl-CoA oxidase were very similar when assayed with the same (common) substrate. Since the two oxidases were purified to a similar extent and with a similar yield, the contribution of each enzyme to substrate oxidation in the column eluate probably reflects its contribution in the intact liver.

The first enzyme of peroxisomal 3-oxidation is an acyl-CoA oxidase (1). In the rat, extrahepatic peroxisomes contain two and liver peroxisomes three acyl-CoA oxidases (2, 3). A first enzyme, palmitoyl-CoA oxidase, oxidizes the CoA esters of straight chain fatty acids. The enzyme is induced in liver and to a lesser extent in some extrahepatic tissues (kidney, intestinal mucosa, heart) by treatment of the animals with peroxisome proliferators (2, 3). Palmitoyl-CoA oxidase has been purified by Osumi et al. (4) and by Inestrosa et al. (5). Its native molecular mass is 150 kDa, and it consists of subunits of 72, 52, and 21 kDa. The latter two subunits are formed in vivo by posttranslational proteolytic cleavage of the 72-kDa subunit (6, 7). The amino acid sequence of the 72-kDa subunit has been reported by Miyazawa et al. (8).

A second enzyme, pristanoyl-CoA oxidase, oxidizes the CoA esters of 2-methyl branched fatty acids such as the synthetic 2-methylpalmitic acid and the naturally occurring pristanic acid, but it also shows activity toward the CoA esters of straight long chain fatty acids (3). The enzyme is not induced by treatment of the animals with peroxisome proliferators (3, 9). Pristanoyl-CoA oxidase has been purified in our laboratory. Its native molecular mass is 420 kDa, and it consists of identical subunits of 70 kDa (3).

Liver peroxisomes contain a third acyl-CoA oxidase, trihydroxyprostanoyl-CoA oxidase, which oxidizes the CoA esters of the bile acid intermediates di- and trihydroxyprostanoic acids (2). The enzyme, which is not induced by peroxisome proliferators, has been partially purified in our laboratory. Its native molecular mass is 139 kDa, and it consists of identical subunits of 69 kDa (2).

Palmitoyl-CoA oxidase, pristanoyl-CoA oxidase, and trihydroxyprostanoyl-CoA oxidase are also present in the human, but the enzymes have been less well characterized than the rat enzymes (10, 11).

Isolated rat liver peroxisomes are capable of 3-oxidizing a wide variety of CoA esters: those of medium, long, and very long chain fatty acids, 2-methyl branched fatty acids, medium and long chain dicarboxylic fatty acids, and the carboxylic side chains of the bile acid intermediates di- and trihydroxyprostanic acids, prostaglandins, and other eicosanoids, and xenobiotics (for reviews, see Refs. 12 and 13). For most of these substrates it is not known by which oxidase(s) they are oxidized. Therefore, we determined the substrate specificity of each of the three acyl-CoA oxidases, using commercially available CoA esters as well as CoA esters that were synthesized in our laboratory.
Partial Purification

Substrate Specificities of Peroxisomal Acyl-CoA Oxidases—Fig. 1 shows the separation of a partially purified preparation of rat liver acyl-CoA oxidases on a DEAE anion-exchange column. In agreement with an earlier report from our laboratory, the inducible acyl-CoA oxidase, which is often called palmitoyl-CoA oxidase, eluted first from the column, followed (in a second peak) by trihydroxypropanoyl-CoA oxidase and (in a third peak) by the non-inducible acyl-CoA oxidase, which we call pristanoyl-CoA oxidase (3). The eluate fractions were analyzed for oxidase activity with different CoA esters as the substrates. The figure shows that the CoA esters and albumin were varied as indicated in the legends to the figures.
substitution resulted in a substrate that was oxidized still predominantly by palmitoyl-CoA oxidase but that was also recognized, albeit poorly, by pristanoyl-CoA oxidase. The CoA esters of long chain mono- and dicarboxylic fatty acids such as palmitoyl-CoA and hexadecanediol-CoA were oxidized by palmitoyl-CoA oxidase but, in addition, a substantial part (~30%) of the total eluate oxidase activity was associated with the fractions containing pristanoyl-CoA oxidase. As the long chain acyl-CoAs, the very long chain lignoceryl-CoA was oxidized both by palmitoyl-CoA oxidase and pristanoyl-CoA oxidase. However, the latter enzyme was now the more active one, catalyzing ~65% of the total eluate activity. The CoA esters of the long chain 2-methyl branched fatty acids, 2-methylpalmitoyl-CoA and pristanoyl-CoA, were oxidized almost completely by pristanoyl-CoA oxidase. A small portion (10% or less) of the overall eluate activity was catalyzed by trihydroxypropanoyl-CoA oxidase. This is perhaps not surprising since the bile acid intermediates di- and trihydroxyxycoprostanic acids possess a branched 2,6-dimethylhexanoic acid side chain. 2-Methylhexanoyl-CoA was oxidized for even more than 50% by trihydroxypropanoyl-CoA oxidase, the remainder of the total eluate activity being associated with the pristanoyl-CoA oxidase peak. Trihydroxypropanoyl-CoA oxidase was oxidized exclusively by trihydroxypropanoyl-CoA oxidase. No oxidase activity was found with isovaleryl-CoA and isobutyryl-CoA, catabolites of leucine and valine, respectively, in either the partially purified enzyme preparation that was loaded on the column or the column eluate.

Identical substrate specificities were observed with purified palmitoyl-CoA oxidase and pristanoyl-CoA oxidase. (Trihydroxypropanoyl-CoA oxidase has not been purified yet.) When a partially purified oxidase preparation from a clofibrate-treated rat was separated on a DEAE column, the same substrate specificities were again detected. As expected, the activities eluting with the first peak (inducible acyl-CoA oxidase) were severalfold increased (data not shown).

Substrate Dependence of Peroxisomal Acyl-CoA Oxidases: Influence of Albumin—The influence of albumin and the substrate dependence in the absence and presence of albumin were studied for each of the oxidases with the acyl-CoA esters used in Fig. 1. The experiments which are presented in detail further in this section, revealed two major points that are worth mentioning at the beginning.

1. In the absence and presence of albumin, the substrate dependence of palmitoyl-CoA oxidase and pristanoyl-CoA oxidase were almost identical for each of their common substrates (palmitoyl-CoA, hexadecanediol-CoA, lignoceryl-CoA): with a particular substrate each enzyme reached its half-maximal activity at a similar substrate concentration; the same was true for the substrate concentrations at which maximal activities were reached. Since in the partially purified enzyme preparation that was loaded on the DEAE column, recoveries of palmitoyl-CoA oxidase and pristanoyl-CoA oxidase were comparable as were their enrichments, the similar substrate dependences allow for an extrapolation of the data of Fig. 1, which were obtained at saturating or near-saturating substrate concentrations, to the in vivo situation where much lower substrate concentrations most probably prevail. This extrapolation suggests that, at least in liver, pristanoyl-CoA oxidase oxidizes a substantial part of the long chain acyl-CoAs and especially of the very long chain acyl-CoAs.

2. Trihydroxypropanoyl-CoA oxidase was hardly active in the absence of albumin, which, when used in the assay, bound most of the trihydroxypropanoyl-CoA. At equal unbound trihydroxypropanoyl-CoA concentrations, trihydroxypropanoyl-CoA oxidase was many times more active in the presence than in the absence of albumin, suggesting that the enzyme may prefer bound trihydroxypropanoyl-CoA over unbound trihydroxypropanoyl-CoA. This may not be a unique feature of trihydroxypropanoyl-CoA oxidase, since also retinoic acid appears to be a better substrate for its catabolizing enzymes, when bound to cellular retinoic acid-binding protein (19).

Figs. 2-19 show the substrate dependences in the absence and presence of albumin (upper panels) and the influence of increasing albumin concentrations at a fixed substrate concentration (lower panels) for palmitoyl-CoA oxidase (Figs. 2-8), pristanoyl-CoA oxidase (Figs. 9-15), and trihydroxypropanoyl-CoA oxidase (Figs. 16-19). The experiments were carried out on pooled DEAE column eluate fractions containing the highest activity of the corresponding enzyme (Fig. 1, fractions 11-13 for palmitoyl-CoA oxidase, fractions 21-23 for pristanoyl-CoA oxidase, fractions 14-16 for trihydroxypropanoyl-CoA oxidase).

The figures reveal that for each enzyme and for each substrate enzymatic activities depend not only on the substrate concentration but also on the albumin concentration and on the substrate/albumin molar ratios and that conditions in which optimum activities are reached differ from enzyme to enzyme and from substrate to substrate. In virtually every instance, albumin was stimulatory, moderately or strongly, when used at the appropriate concentration.

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2 Oxidase activities were also measured with octanoyl-CoA (200 μM), decanoyl-CoA (175 μM), and lauryl-CoA (150 μM) in fractions 12 and 21, containing the highest palmitoyl-CoA oxidase activity and pristanoyl-CoA oxidase activity, respectively. The ratios of activities found in fraction 12 (palmitoyl-CoA oxidase) over those found in fraction 21 (pristanoyl-CoA oxidase) were 3.7, 2.1, and 3.9 for octanoyl-CoA, decanoyl-CoA, and lauryl-CoA, respectively, as compared to 2.8 for palmitoyl-CoA. These results indicate that also the CoA esters of medium chain fatty acids are oxidized partly by pristanoyl-CoA oxidase.

3 In an earlier study we did not find activity of trihydroxypropanoyl-CoA oxidase toward 2-methylpalmitoyl-CoA or pristanoyl-CoA (3). This is related to the assay conditions. In these earlier experiments 2-methylpalmitoyl-CoA oxidase and pristanoyl-CoA oxidase were measured in the absence of albumin, a condition in which trihydroxypropanoyl-CoA oxidase is hardly active (see further).
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Fig. 3. Hexadecanediyl-CoA dependence of palmitoyl-CoA oxidase: influence of albumin. Palmitoyl-CoA oxidase was measured in fractions 11-13 from the DEAE column (Fig. 1) with hexadecanediyl-CoA as the substrate. Upper panel, hexadecanediyl-CoA dependence in the absence (A) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 50 μM hexadecanediyl-CoA.

Theoretically, the stimulatory effect of albumin could be due to several factors or to a combination of these factors: stabilization or protection from inactivation of the enzyme, release of substrate inhibition by binding excess substrate, enhancement of product dissociation from the enzyme by product binding, and preference by the enzyme of bound over unbound substrate.

Palmitoyl-CoA oxidase displayed marked substrate inhibition at higher concentrations of palmitoyl-CoA (Fig. 2) and lignoceroyl-CoA (Fig. 4). The stimulatory effect of albumin at these elevated substrate concentrations may be partly the result of binding of the excess CoA esters. This cannot be the only explanation, however. Since albumin contains six to seven binding sites for palmitoyl-CoA (18), its binding capac-

Fig. 5. Prostaglandin E2-CoA dependence of palmitoyl-CoA oxidase: influence of albumin. Palmitoyl-CoA oxidase was measured in fractions 11-13 from the DEAE column (Fig. 1) with prostaglandin E2-CoA as the substrate. Upper panel, prostaglandin E2-CoA dependence in the absence (A) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 50 μM prostaglandin E2-CoA.

Fig. 6. 6-Phenylhexanoyl-CoA dependence of palmitoyl-CoA oxidase: influence of albumin. Palmitoyl-CoA oxidase was measured in fractions 11-13 from the DEAE column (Fig. 1) with 6-phenylhexanoyl-CoA as the substrate. Upper panel, 6-phenylhexanoyl-CoA dependence in the absence (A) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM 6-phenylhexanoyl-CoA.
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Fig. 7. Hexanoyl-CoA dependence of palmitoyl-CoA oxidase: influence of albumin. Palmitoyl-CoA oxidase was measured in fractions 11–13 from the DEAE column (Fig. 1) with hexanoyl-CoA as the substrate in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. The stimulatory effect of albumin did not vary over a concentration range of 0.01–0.5% (w/v); hexanoyl-CoA concentration, 100 μM (data not shown).

Fig. 8. Glutaryl-CoA dependence of palmitoyl-CoA oxidase: influence of albumin. Palmitoyl-CoA oxidase was measured in fractions 11–13 from the DEAE column (Fig. 1) with glutaryl-CoA as the substrate in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. The stimulatory effect of albumin did not vary over a concentration range of 0.01–0.5% (w/v); glutaryl-CoA concentration, 100 μM (data not shown).

Fig. 9. Pristanoyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with pristanoyl-CoA as the substrate. Upper panel, pristanoyl-CoA dependence in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM pristanoyl-CoA.

Fig. 10. 2-Methylpalmitoyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with 2-methylpalmitoyl-CoA as the substrate. Upper panel, 2-methylpalmitoyl-CoA dependence in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM 2-methylpalmitoyl-CoA.

ity, when used at a concentration of 9 μM (Fig. 2, upper panel), does not seem to suffice to explain the stimulatory effect observed at palmitoyl-CoA concentrations higher than 100 μM. At low concentrations of long and very long chain acyl-CoAs albumin became inhibitory, most likely by binding the CoA esters, which would result in a lowering of the unbound substrate concentration. With the more polar substrates, which did not induce substrate inhibition, the stimulatory effect of albumin was less dramatic and also present at low substrate concentrations (Figs. 5–8). The reason for this stimulation remains unclear.

With pristanoyl-CoA oxidase, long (straight and branched) and very long chain acyl-CoA esters caused much less substrate inhibition at elevated concentrations (Figs. 9–13) and the stimulatory effect of albumin was also less substantial than that seen with palmitoyl-CoA oxidase. At lower concentrations of these CoA esters albumin became again inhibitory. As was the case for palmitoyl-CoA oxidase, it cannot be deduced from our experiments why albumin stimulated pristanoyl-CoA oxidase with the more polar substrates, which did not cause substrate inhibition, and why this stimulation was particularly marked at low substrate concentrations (see Fig. 14).

For palmitoyl-CoA oxidase as well as pristanoyl-CoA oxidase, apparent $K_m$ values increased with decreasing chain length and/or increasing polarity of the substrates. For each of the substrates that are shared by palmitoyl-CoA oxidase and pristanoyl-CoA oxidase (palmitoyl-CoA, hexadecane-
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FIG. 11. Palmitoyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with palmitoyl-CoA as the substrate. Upper panel, palmitoyl-CoA dependence in the absence (▲) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 100 μM palmitoyl-CoA.

FIG. 12. Hexadecanediol-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with hexadecanediol-CoA as the substrate. Upper panel, hexadecanediol-CoA dependence in the absence (▲) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 50 μM hexadecanediol-CoA.

FIG. 13. Lignoceroyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with lignoceroyl-CoA as the substrate. Upper panel, lignoceroyl-CoA dependence in the absence (▲) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM lignoceroyl-CoA. All assays (upper and lower panels) also contained 525 μM β-cyclodextrin. Assay mixtures without albumin became cloudy at lignoceroyl-CoA concentrations above 100 μM.

FIG. 14. 2-Methylhexanoyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with 2-methylhexanoyl-CoA as the substrate. Upper panel, 2-methylhexanoyl-CoA dependence in the absence (▲) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 225 μM 2-methylhexanoyl-CoA.

oxidase is hardly active in the absence of albumin (Figs. 16–19). Although substrate inhibition was observed with some of the substrates, the magnitude of the stimulatory effect makes it doubtful that a mere release of substrate inhibition was...

diol-CoA, lignoceroyl-CoA), substrate dependences in the absence and presence of albumin were comparable for palmitoyl-CoA oxidase and pristanoyl-CoA oxidase (compare Figs. 2 and 11, Figs. 3 and 12, Figs. 4 and 13) indicating that the affinity of the enzymes for a particular common substrate is similar.

A surprising finding was that trihydroxycoprostanoyl-CoA...
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FIG. 15. 6-Phenylhexanoyl-CoA dependence of pristanoyl-CoA oxidase: influence of albumin. Pristanoyl-CoA oxidase was measured in fractions 21–23 from the DEAE column (Fig. 1) with 6-phenylhexanoyl-CoA as the substrate. Upper panel, 6-phenylhexanoyl-CoA dependence in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM 6-phenylhexanoyl-CoA.

FIG. 16. Trihydroxycoprostanoyl-CoA dependence of trihydroxycoprostanoyl-CoA oxidase: influence of albumin. Trihydroxycoprostanoyl-CoA oxidase was measured in fractions 14–16 from the DEAE column (Fig. 1) with trihydroxycoprostanoyl-CoA as the substrate. Upper panel, trihydroxycoprostanoyl-CoA dependence in the absence (△) and presence (●) or 36 μM (0.24%, w/v) albumin (○) or 36 μM (0.24%, w/v) albumin (○). Lower panel, albumin dependence in the presence of 75 μM trihydroxycoprostanoyl-CoA.

FIG. 17. 2-Methylhexanoyl-CoA dependence of trihydroxycoprostanoyl-CoA oxidase: influence of albumin. Trihydroxycoprostanoyl-CoA oxidase was measured in fractions 14–16 from the DEAE column (Fig. 1) with 2-methylhexanoyl-CoA as the substrate. Upper panel, 2-methylhexanoyl-CoA dependence in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 225 μM 2-methylhexanoyl-CoA.

FIG. 18. Pristanoyl-CoA dependence of trihydroxycoprostanoyl-CoA oxidase: influence of albumin. Trihydroxycoprostanoyl-CoA oxidase was measured in fractions 14–16 from the DEAE column (Fig. 1) with pristanoyl-CoA as the substrate. Upper panel, pristanoyl-CoA dependence in the absence (△) and presence (●) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM pristanoyl-CoA.

(The only) cause of the stimulation. The phenomenon was investigated in more detail with trihydroxycoprostanoyl-CoA, the natural substrate for trihydroxycoprostanoyl-CoA oxidase. The enzyme was not irreversibly inactivated during the assay in the absence of albumin, since addition of albumin to an assay mixture containing enzyme and substrate and preincubated in the absence of albumin, restored full activity (data not shown). In another experiment, we investigated whether albumin binds trihydroxycoprostanoyl-CoA. Fig. 20 shows that at trihydroxycoprostanoyl-CoA concentrations below 70 μM more than 90% of the trihydroxycoprostanoyl-CoA was...
presence in the absence of albumin. This suggests that
prostanoyl-CoA oxidase was many times more active in the
trihydroxycoprostanoyl-CoA oxidase prefers bound trihy-
trihydroxycoprostanoyl-CoA were separated by ultrafiltration (Centrifree
portion of the long chain acyl-CoAs (20–23), whereas perox-
tropic acid is oxidized almost completely by pristanoyl-CoA
oxidase. Since the apparent
oxidase.
be oxidized to any large extent
short chain straight acyl-CoAs and short chain dicarboxylic fatty acids (28–30), 2-methyl branched fatty acids
(9), prostaglandins (16, 31), and possibly other eicosanoids
(32). The CoA esters of the bile acid intermediates di- and trihydroxycoprostanic acids are oxidized solely by peroxi-
somes; they are not substrates for mitochondria (33–35).

Our results show that among the physiological substrates for peroxisomes (i.e., those substrates that are oxidized exclu-
sively or predominantly by peroxisomes in the intact cell) the
CoA esters of prostaglandins and those of the bile acid inter-
mediates are oxidized exclusively by palmitoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase, respectively. The
CoA ester of the isoprenoid-derived 2-methyl branched prista-
nic acid is oxidized almost completely by pristanoyl-CoA
oxidase. Trihydroxycoprostanoyl-CoA oxidase also shows
some activity with this substrate but it is low. Because of this
low activity and because trihydroxycoprostanoyl-CoA oxidase
is absent from extrahepatic tissues (3), it is doubtful that this
enzyme plays a significant role in the degradation of long
chain isoprenoid-derived fatty acids in vivo. Short chain 2-
methyl branched fatty acids, which may originate from the
breakdown of long chain isoprenoid-derived fatty acids, are
perhaps better substrates for trihydroxycoprostanoyl-CoA ox-
idase than for pristanoyl-CoA oxidase. Since the apparent K_m
values of the peroxisomal oxidases appear to be inversely
related to the chain length of the substrates, it is again
doubtful that short chain branched acyl-CoAs (as well as short
chain straight acyl-CoAs and short chain dicarboxylyl-
CoAs such as glutaryl-CoA) are degraded to any large extent
by peroxisomes in vivo. This contention agrees with the
general belief that peroxisomal β-oxidation does not go to
completion but that it rather acts as a chain-shortening

DISCUSSION

As mentioned in the Introduction and as confirmed in this report, peroxisomes are capable of β-oxidizing a wide variety
of acyl-CoA derivatives. The substrate spectrum of peroxi-
somes partly overlaps that of the mitochondria, which are
also capable of oxidizing a broad range of CoA esters. Al-
though mitochondria and peroxisomes share a number of
substrates, the contribution of each organelle to the oxidation
of these common substrates is not equally important in the
intact cell. Available evidence indicates that in the intact cell
or in the intact organism mitochondria oxidize the major
portion of the long chain acyl-CoAs (20–23), whereas perox-
isomes are responsible for the oxidation of the major portion
of the CoA esters of very long chain fatty acids (24–27),
dicarboxylic fatty acids (28–30), 2-methyl branched fatty acids
(9), prostaglandins (16, 31), and possibly other eicosanoids
(32). The CoA esters of the bile acid intermediates di- and trihydroxycoprostanic acids are oxidized solely by peroxi-
somes; they are not substrates for mitochondria (33–35).

It should be noted that this statement is true only for animal
cells. In plants and eukaryotic microorganisms, peroxisomes (or glyoxysomes) are the only site of β-oxidation.

bound at the albumin concentration used in the assay. Com-
parison of Figs. 16 and 20 reveals that at equal unbound
trihydroxycoprostanoyl-CoA concentrations trihydroxycopro-
stanoyl-CoA oxidase was many times more active in the
presence than in the absence of albumin. This suggests that
trihydroxycoprostanoyl-CoA oxidase prefers bound trihy-

Fig. 19. 2-Methylpalmitoyl-CoA dependence of trihydroxycoprostanoyl-CoA oxidase: influence of albumin. Trihydroxycoprostanoyl-CoA oxidase was measured in fractions 14–16 from the DEAE column (Fig. 1) with 2-methylpalmitoyl-CoA as the substrate. Upper panel, 2-methylpalmitoyl-CoA dependence in the absence (A) and presence (O) of 9 μM (0.06%, w/v) albumin. Lower panel, albumin dependence in the presence of 75 μM 2-methylpalmitoyl-CoA.

Fig. 20. Trihydroxycoprostanoyl-CoA binding to albumin. [14C]Trehydroxycoprostanoyl-CoA (specific radioactivity, 5.7 μCi/μmol), synthesized as described before (15), was added to 40 mM potassium phosphate buffer, pH 8.3, containing 0.24% (w/v) albumin (conditions used in the trihydroxycoprostanoyl-CoA oxidase assay) at the concentrations indicated in the figure. After 30 min of equili-
bration, an aliquot of the mixtures was counted for radioactivity (total concentration), and unbound and bound trihydroxycopro-
stanoyl-CoA were separated by ultrafiltration (Centrifree microparti-
tion system, Amicon, Danvers, MA) of the remainder of the mixtures. An aliquot of the ultrafiltrates was counted for radioactivity (unbound concentration).

It should be noted that this statement is true only for animal
cells. In plants and eukaryotic microorganisms, peroxisomes (or glyoxysomes) are the only site of β-oxidation.
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system (see Refs. 12 and 13). As long chain acyl-CoAs, long chain dicarboxyl-CoAs appear to be oxidized partly by palmitoyl-CoA oxidase and partly by pristanoyl-CoA oxidase, the former enzyme being the more active one. For the oxidation of very long chain acyl-CoAs, pristanoyl-CoA oxidase seems to be more important, at least in liver, than palmitoyl-CoA oxidase.

Our observations raise a number of questions with regard to the molecular defects in certain peroxisomal diseases. Is it possible, if the same substrate specificities occur in the human, that some cases of X-linked adrenoleukodystrophy are the result of a pristanoyl-CoA oxidase deficiency? X-Linked adrenoleukodystrophy, a disease that affects mainly the central nervous system and the adrenals, is characterized by an accumulation of very long chain fatty acids in tissues and body fluids and is believed to be caused by a deficiency of a peroxisomal very long chain acyl-CoA synthetase (36). Biochemically, X-linked adrenoleukodystrophy is diagnosed by measuring very long chain fatty acid levels. In view of our results, it might be of importance to also measure pristane acid levels.

On the other hand, the few cases of isolated palmitoyl-CoA oxidase deficiency that have been described also present with elevated very long chain fatty acid levels (37, 38). This suggests that pristanoyl-CoA oxidase alone does not suffice to handle the organism’s very long chain fatty acid load or that the latter enzyme is less active toward very long chain fatty acids in the human. Clearly, the determination of the activities of palmitoyl-CoA oxidase and pristanoyl-CoA oxidase in different human tissues and the determination of the enzymes’ substrate specificities are needed before these questions can be answered.

In our experiments glutaryl-CoA appeared to be oxidized by palmitoyl-CoA oxidase and not by a separate enzyme (39). In agreement with this idea, the glutaryl-CoA oxidase activity copurified with palmitoyl-CoA oxidase during the further purification of the enzyme to homogeneity, confirming earlier findings by Poosch and Yamazaki (40). Our findings are difficult to reconcile with the recent description of a glutaryl-CoA oxidase deficiency in a patient with glutaric aciduria, who had a seemingly normal palmitoyl-CoA oxidase activity (41). However, we cannot exclude the possibility of the existence of a separate glutaryl-CoA oxidase that was lost or inactivated in the early purification steps of our enzyme preparation.

A surprising finding of the present study is the strong albumin dependence of the partially purified trihydroxyco- prostanoyl-CoA oxidase. One possible explanation that needs further investigation is that the enzyme prefers bound substrate. Is it possible that in the intact cell sterol carrier protein-2, which is highly concentrated in the peroxisomal matrix (42, 43), plays a role in binding the CoA esters of the bile acid intermediates? This question may be related to the unexplained findings in a patient whom we recently described (44). This patient was suspected to suffer from a trihydroxycoprostanoyl-CoA synthetase or a trihydroxyco- prostanoyl-CoA oxidase deficiency on clinical-chemical grounds (elevated plasma levels of di- and trihydroxycoprostanic acids, normal plasma levels of very long chain fatty acids). Trihydroxyco- prostanoyl-CoA synthetase and trihydroxyco- prostanoyl-CoA oxidase were normal as was the oxidation of [2-14C]trihydroxyco- prostanoyl-CoA in liver tissue from the patient (44). These findings suggest that some other hitherto unknown factor, possibly a binding or transport protein, was deficient. We could not detect oxidase activity with isovaleryl-CoA and isobutyryl-CoA, catabolites of leucine and valine, respectively. This suggests that, in contrast with certain peroxisomes from plants (45, 46), mammalian peroxisomes do not contain oxidases involved in the catabolism of branched amino acids.

Finally, some general conclusions can be drawn with regard to the chemical characteristics of the acyl-CoA oxidase substrates. Palmitoyl-CoA oxidase appears to oxidize only the CoA esters of straight chain fatty acids and of other molecules that possess an unbranched carboxyl side chain (e.g. 6-phenylhexanoic acid, prostaglandin E2). The apparent Km of the enzyme increases with decreasing chain length and/or increasing polarity (e.g. prostaglandin E2) of the substrate. Modification of the ω-ω and ω-substitutions do not seem to affect the enzyme’s activity in a dramatic way. Pristanoyl- CoA oxidase oxidizes the CoA esters of short as well as 2-methyl branched fatty acids. Shorter and/or more polar molecules are poor (branched molecules) or no (straight molecules) substrates for the enzyme. Trihydroxyco- prostanoyl-CoA oxidase recognizes only 2-methyl branched substrates. Because oxidizing the bile acid intermediates di- and trihydroxycoprostanic acids, the enzyme is capable of oxidizing short as well as long chain 2-methyl branched fatty acids. Pristanoyl-CoA oxidase recognizes both optical isomers of its 2-methyl branched substrates, since excess enzyme completely desaturates limiting amounts of the CoA esters of synthetically prepared 2-methylpalmitic and pristanic acids.5 In this regard, it resembles trihydroxyco- prostanoyl-CoA oxidase, which also does not discriminate between the R and S isomers of trihydroxyco- prostanoyl-CoA (33).

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