Function and dysfunction of the mitochondrial and peroxisomal beta-oxidation systems in human cells and their functional interaction

Jakobs, B.S.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE (Digital Academic Repository)
CHAPTER 5

IMPAIRED PEROXISOMAL FATTY ACID OXIDATION OBSERVED IN MITOCHONDRIAL ACYL-CARNITINE/CARNITINE TRANSLOCASE DEFICIENT CELLS:

The first evidence for the involvement of carnitine in shuttling propionyl-CoA from peroxisomes to mitochondria.


SUMMARY
We have investigated how [1-14C]propionyl-CoA, the first product of the peroxisomal β-oxidation of [1-14C]pristanate, is transported to mitochondria for further oxidation in human skin fibroblasts from patients with a defect in the mitochondrial carnitine/acylcarnitine translocase and CPT II, respectively. Oxidation of pristanate was found to be partially deficient in both types of mutant cells. More important, 14CO2 production was completely deficient in the carnitine/acylcarnitine translocase deficient cells but not in the CPT II deficient cells. These results strongly suggest that formation of 14CO2 in the Krebs-cycle from [1-14C]propionyl-CoA as generated in peroxisomes requires the active participation of the mitochondrial carnitine/acylcarnitine translocase. The results described in this paper provide the first evidence indicating that propionyl-CoA leaves the peroxisome as a carnitine ester and strongly suggest that the commonly accepted concept that peroxisomal β-oxidation is not dependent on carnitine is incorrect.

INTRODUCTION
In 1976 Lazarow and De Duve discovered that peroxisomes, like mitochondria, are capable of β-oxidizing fatty acids. Despite the increased knowledge on the kind of fatty acids and fatty acid derivatives that are chain-shortened in peroxisomal β-oxidation and the enzymes involved (see for review [Reddy and Mannaerts, 1994]), little is known concerning the functional characteristics of the peroxisomal β-oxidation system. First, it is unclear how fatty acids or fatty acyl-CoA esters enter the peroxisome. Available evidence suggests that fatty acids transport across the peroxisomal membrane is not dependent on carnitine [Thomas et al., 1980; Appelkvist and Dallner, 1980] in contrast to the situation in mitochondria [McGarry et al., 1992]. Second, there is no information on the question how NADH, generated at the third step of peroxisomal β-oxidation, is re-oxidized. In addition, it is unknown how the endproducts of peroxisomal β-oxidation, which include acetyl-CoA, propionyl-CoA and medium-chain acyl-CoA esters are transported out of the peroxisomes to mitochondria for final oxidation. In principle there are 3 possibilities: first, the CoA-esters may leave the peroxisome as such; second, they may leave the peroxisome as free acids after hydrolysis by one of a variety of acyl-CoA hydrolases or third, the acyl-CoA esters are transformed into the corresponding acyl-carnitine esters by one of the carnitine-acyltransferases present in peroxisomes. At least two transferases, i.e. COT [Miyazawa et al., 1983] and CAT [Markwell et al., 1973] have been reported to be present in peroxisomes, both localized in the matrix. Farrell and Bieber [1983] suggested that these two enzymes might be involved in transport of the products of peroxisomal β-oxidation (acetyl-CoA, propionyl-CoA and medium acyl-CoA esters) from peroxisomes to the mitochondria as carnitine esters for further oxidation. On the other hand, studies by Leighton et al. [1989] have shown that the acetyl-CoA units produced during peroxisomal β-oxidation in rat hepatocytes are predominantly released as free acetate.

The studies described in this paper were carried out in order to obtain information on the question how the first product of the peroxisomal β-oxidation of [1-C14]pristanate, i.e. [1-C14]propionyl-CoA is transported from peroxisomes to mitochondria for final oxidation. For this purpose, we used cultured human skin fibroblasts from patients with certain defined deficiencies at the level of the transport of fatty acids across the mitochondrial membrane. The results obtained show that carnitine is involved in transporting propionyl-CoA to mitochondria for
final oxidation to $\text{CO}_2$ and $\text{H}_2\text{O}$. These findings shed new light on the role of carnitine in fatty acid $\beta$-oxidation and should lead to a reevaluation of the commonly accepted concept (see [Reddy and Mannaerts, 1994], for review) that peroxisomal $\beta$-oxidation is independent of carnitine.

MATERIALS AND METHODS

Cell lines and culture conditions:
Cultured human skin fibroblasts were obtained from control subjects and patients with a deficiency of carnitine/acyl carnitine translocase [Pande et al., 1993] or CPT II [Demaugre et al., 1991]. Human skin fibroblasts were grown under 5% $\text{CO}_2$ in HAM's F-10 medium (Gibco, Paisley, Scotland), supplemented with 15% Hyclone bovine calf serum (Hyclone, Logan, U.S.A) plus antibiotics. At confluence, the fibroblasts were harvested by trypsinisation according to standard procedures. The fibroblasts were resuspended in HAMs F-10 and kept at room temperature until further use.

Preparation of substrates for $\beta$-oxidation assays:
Stock solutions of [1-$^{14}$C]octanoate, [1-$^{14}$C]palmitate and [1-$^{14}$C]pristanate at concentrations of 2 mM, 2 mM and 100 $\mu$M, respectively, were prepared as described before [Jakobs et al., 1994].

Measurement of fatty acid $\beta$-oxidation:
Fatty acid $\beta$-oxidation activities were measured in fibroblasts grown overnight in glass vials according to [Jakobs et al., 1994] with minor modifications as described here. The reaction medium consisting of RPMI (Gibco, Paisley, Scotland) lacking glucose and glutamine but supplemented with 2 mM l-carnitine and 20 mM HEPES pH 7.4 was added in a final volume of 500 $\mu$L. Fatty acid oxidation was initiated by the addition of 50 $\mu$L [1-$^{14}$C]octanoate, palmitate or pristanate (at final concentrations of 200 $\mu$M, 200 $\mu$M and 10 $\mu$M, respectively) and allowed to proceed for 2 h at 37°C. Reactions were terminated with 100 $\mu$L 2.6 M PCA and the $\beta$-oxidation activities were measured as the sum of the amount of $^{14}$CO$_2$ released and the amount of [1-$^{14}$C]water-soluble $\beta$-oxidation products formed as described before [Wanders et al., 1987b]. [1-$^{14}$C]water-soluble products formed during octanoate $\beta$-oxidation were determined on a 6 ml Bakerbond extraction column packed with reversed phase octadecylsilane (C18) bonded to silicagel (40 $\mu$m APD, 60 Å). The column was equilibrated with 3 ml methanol and 3 ml 50 mM sodium acetate pH 3.0, after which the reaction mixture was put on top of the column and eluted twice with 1 ml methanol. Fractions were collected in glass vials and counted in scintillation medium.

Materials:
Culture media were purchased from Gibco (Paisley, Scotland). Hyclone bovine calf serum was obtained from Hyclone (Logan, U.S.A). Octanoate and palmitate were purchased from Sigma Chemie (Bornem, Belgium). [1-$^{14}$C]octanoate and [1-$^{14}$C]palmitate were obtained from NEN (s'Hertogenbosch, The Netherlands). Bakerbond extraction columns were obtained from J.T. Baker Inc. (Phillipsburg, USA).

RESULTS

Fatty acid $\beta$-oxidation in control and mutant fibroblasts with a deficiency of either the mitochondrial carnitine/acylcarnitine translocase or CPT II.

In the experiment of Table 1 we studied the $\beta$-oxidation of three different [1-$^{14}$C]labelled substrates octanoate, palmitate and pristanate in control and mutant fibroblasts with an established deficiency of the mitochondrial carnitine/acylcarnitine translocase [Pande et al., 1993] or CPT II [Demaugre et al., 1991]. The results show that oxidation of octanoate is normal in both types of mutant cells. This is in line with the notion that octanoate enters the mitochondrion in a carnitine independent fashion, as the free acid itself (for review see [Guzmán and Geelen, 1993; Reddy and Mannaerts, 1994]). Palmitate oxidation, however, was found to be deficient in these cells.
Table 1. Production of $^{14}$CO$_2$ plus [1-$^{14}$C]water-soluble products during oxidation of [1-$^{14}$C]octanoate, [1-$^{14}$C]palmitate and [1-$^{14}$C]pristanate in human skin fibroblasts from control subjects and patients with a deficiency of the carnitine/acylcarnitine translocase or CPT II.

<table>
<thead>
<tr>
<th>Type of fibroblasts studied</th>
<th>Rate of fatty acid oxidation (nmol*h$^{-1}$*mg protein$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Octanoate</td>
</tr>
<tr>
<td>Control</td>
<td>1.03 ±0.57 (12)</td>
</tr>
<tr>
<td>Translocase def.</td>
<td>0.88 ±0.46 (10)</td>
</tr>
<tr>
<td>CPT II def.</td>
<td>1.11 ±0.43 (6)</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± S.D. with the number of different experiments between parentheses.

This result is in accordance with the established fact that long-chain fatty acids require the carnitine translocation system for transfer across the mitochondrial membrane before oxidation can occur within the mitochondrial matrix.

Table 1 further shows that oxidation of [1-$^{14}$C]pristanate was reduced to about 25% of control values in the mitochondrial carnitine/acylcarnitine translocase deficient cells. Since the carnitine/acylcarnitine translocase and CPT II are functionally coupled, we also studied [1-$^{14}$C]pristanate β-oxidation in a CPT II deficient cell line (Table 1). [1-$^{14}$C]Pristanate β-oxidation was found to be reduced in these cells (about 70% of control levels).

Figure 1. The production of $^{14}$CO$_2$ from control subjects (A), carnitine/acylcarnitine translocase deficient cells (B) or CPT II deficient cells (C) incubated with [1-$^{14}$C]pristanate relative to the total production of $[^{14}$CO$_2$ plus $[^{14}$C]water-soluble products.

Ratios are expressed as the amount of $^{14}$CO$_2$ released divided by the total amount of [1-$^{14}$C]β-oxidation products ($^{14}$CO$_2$ plus $[^{14}$C]acid-soluble products) formed during β-oxidation of [1-$^{14}$C]pristanic acid. Ratios are multiplied by 1000.
\(^{14}\text{CO}_2\) production from [1-\(^{14}\text{C}\)]pristanate is strongly reduced in cells deficient in the mitochondrial carnitine/acylcarnitine translocase

In the experiment of Table 1 \(\beta\)-oxidation activity was measured as the sum of \(^{14}\text{CO}_2\) and [1-\(^{14}\text{C}\)]water-soluble \(\beta\)-oxidation products. Close inspection of the results obtained with the carnitine/acylcarnitine translocase deficient cells, however, revealed that the formation of \(^{14}\text{CO}_2\) was much more reduced as compared to the production of [1-\(^{14}\text{C}\)]water-soluble \(\beta\)-oxidation products. This is best illustrated if \(^{14}\text{CO}_2\) production is expressed relative to the total production of \(^{14}\text{CO}_2\) plus [1-\(^{14}\text{C}\)]water-soluble \(\beta\)-oxidation products (Fig. 1). Although \(\beta\)-oxidation of [1-\(^{14}\text{C}\)]pristanate was decreased in CPT II deficient cells, the ratio \(^{14}\text{CO}_2\) over total [1-\(^{14}\text{C}\)]\(\beta\)-oxidation products was only partially decreased as compared to control cells and carnitine/acylcarnitine translocase deficient cells (Fig. 1).

**DISCUSSION**

In this paper we have used [1-\(^{14}\text{C}\)]pristanate as a model substrate to study the fate of one of the products of \(\beta\)-oxidation in peroxisomes, i.e. propionyl-CoA. Earlier studies [Jakobs et al., 1994; Singh H et al., 1990] have shown that at least the first cycle of \(\beta\)-oxidation of pristanic acid is taking place exclusively in peroxisomes. In principle there are three ways in which the [1-\(^{14}\text{C}\)]propionyl-CoA produced in peroxisomes can be transported to the mitochondria for final oxidation to \(^{14}\text{CO}_2\) in the Krebs-cycle. The first possibility, as a propionyl-CoA, is highly unlikely due to the fact that the mitochondrial membrane is impermeable for CoA-esters. The second possibility would be that propionyl-CoA is hydrolysed by a NADH-dependent hydrolase [Alexson et al., 1989] in the peroxisomal matrix to CoASH and propionic acid followed by transfer of propionic acid across the peroxisomal membrane towards the mitochondria. Just like acetic acid, propionic acid can easily pass membranes in the protonated form. The third possible mechanism is propionyl-CoA is converted to propionyl-carnitine within peroxisomes via one of the carnitine-acyltransferases (COT, CAT) [Miyazawa et al., 1983; Markwell et al., 1973] followed by transport to the interior of the mitochondria via the mitochondrial carnitine/acylcarnitine translocase followed by retroconversion of propionyl-carnitine to propionyl-CoA. Once inside the mitochondria propionyl-CoA is converted into succinyl-CoA followed by combustion to \(\text{CO}_2\) and \(\text{H}_2\text{O}\) in the Krebs-cycle via the consecutive action of propionyl-CoA carboxylase, methylmalonyl-CoA racemase and L-methylmalonyl-CoA mutase. Bieber and coworkers [Farrell et al., 1984] have purified a short-chain-acyl-CoA specific carnitine-acyltransferase from mouse liver peroxisomes which was found to show highest activity with acetyl-CoA, propionyl-CoA and butyryl-CoA. Furthermore it is known that the mitochondrial carnitine/acylcarnitine carrier possesses a broad chain length specificity accepting a wide variety of acyl-carnitines [Murthy and Pande, 1984].

The results described in this paper show that \(^{14}\text{CO}_2\) formation from [1-\(^{14}\text{C}\)]pristanate is virtually completely deficient in cells from a patient with an established deficiency of the mitochondrial carnitine/acylcarnitine carrier. These results suggest strongly that the third mechanism is operative at least in fibroblasts (Fig 2.). After import into the mitochondria the propionyl-carnitine is converted back into propionyl-CoA via mitochondrial carnitine-
acetyltransferase which also accepts propionyl-CoA as substrate as demonstrated by diDonato and coworkers [Bloisi et al., 1990]. CPT II may also contribute based on the findings of a lowered rate of $^{14}$CO$_2$ formation from [1-$^{14}$C]pristanate in CPT II deficient fibroblasts (fig. 1).

Figure 2. Proposed model for the functional interaction between mitochondria and peroxisomes in cultured human skin fibroblasts to oxidize propionyl-CoA, produced during peroxisomal β-oxidation of [1-$^{14}$C]pristanate, to CO$_2$ in mitochondria.

Abbreviations are: CAT; carnitine-acetyltransferase, (D)MM-CoA; (D)methylmalonyl-CoA, (L)MM-CoA; (L)methylmalonyl-CoA, succ-CoA; succinyl-CoA, IN;inner membrane, OM; outer membrane, TR; carnitine/acylcarnitine translocase, CPT II; carnitine-palmitoyltransferase II. The numbers denote the following: propionyl-CoA carboxylase (1); methylmalonyl-CoA racemase (2); L-methylmalonyl-CoA mutase (3).

Earlier Leighton and coworkers [1989] showed that a considerable proportion of the acetyl-CoA produced during peroxisomal β-oxidation in hepatocytes is transformed into acetate. This suggests that the second mechanism of removal of acetyl-CoA and/or propionyl-CoA from peroxisomes may be much more active in liver as compared to fibroblasts due to, for instance, a much higher short-chain acyl-CoA hydrolase activity in liver. This may well have to do with the different functions of the peroxisomal β-oxidation system in liver and fibroblasts. The liver is a central organ in distributing end-products of β-oxidation to other tissues. This includes 3-hydroxybutyrate and acetoacetate as the products of mitochondrial β-oxidation but may also include acetate, produced during peroxisomal β-oxidation, which may be transported to peripheral tissues followed by activation to acetyl-CoA and combustion in the Krebs-cycle. In other tissues than liver it would seem logical if the end products of peroxisomal β-oxidation are shuttled to the mitochondria for energy purposes rather than allowing the acetyl-CoA to escape from the cell as acetate.
An interesting aspect of the results described in this paper is that overall pristanate β-oxidation is impaired in the cells with a translocase deficiency (Table 1). One explanation for this finding may be that such a deficiency leads to elevated levels of propionyl-carnitine and propionyl-CoA in the cytosol and peroxisomes giving rise to product-inhibition of the peroxisomal β-oxidation system for instance at the level of peroxisomal 3-oxoacyl-CoA thiolase. It has been shown that acetyl-CoA is a strong inhibitor of this enzyme competitive with free CoASH and uncompetitive with 3-ketoacyl-CoA esters. The same may be true for propionyl-CoA although this was not tested by Miyazawa et al. [1981].

ACKNOWLEDGEMENTS

The authors are grateful to Dr Barbara Garavaglia, Dr Jean Paul Bonnefont and Dr Michèle Brivet for allowing us to study fibroblasts from their patients. We thank Mrs Ellen de Jong-Meijboom and Mrs Patricia Veltman for growth and maintenance of cell cultures. We further thank the Stichting Klinische Genetica Amsterdam for financial support.