

β -oxidation of polyunsaturated fatty acids in peroxisomes

Subcellular distribution of Δ^3, Δ^2 -enoyl-CoA isomerase activity in rat liver

T. Kärki*, E. Hakkola*, I.E. Hassinen* and J.K. Hiltunen*⁺

Departments of *Medical Biochemistry and ⁺Clinical Chemistry, University of Oulu, Kajaanintie 52A, SF-90220 Oulu, Finland

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The metabolism of the double bonds at the Δ^3 position in fatty acids was studied in rat liver. Infusion of Δ^3 -*trans*-dodecenoic acid into isolated perfused liver and subcellular fractionation studies showed the presence of both peroxisomal and mitochondrial Δ^3, Δ^2 -enoyl-CoA isomerase activity (EC 5.3.3.8). These findings together with the previous demonstration of peroxisomal 2,4-dienoyl-CoA reductase (EC 1.3.1.34) [(1981) *J. Biol. Chem.* 256, 8259–8262] and D-3-OH-acyl-CoA epimerase (EC 5.1.2.3) [(1985) *FEBS Lett.* 185, 129–134] activities show that peroxisomes possess all the auxiliary enzymes required for the β -oxidation of unsaturated fatty acids.

Polyunsaturated fatty acid; Peroxisome; Catalase compound I; Organ spectroscopy; (Rat liver)

1. INTRODUCTION

Very-long-chain fatty acids accumulate in patients with deficient peroxisomal β -oxidation as a consequence of an inherited disease [1–4]. This fact together with the *in vitro* characteristics of peroxisomal β -oxidation indicates that the physiological task of this metabolic process is the catabolism of long-chain fatty acids [5]. Long-chain fatty acids in nature are often unsaturated, however, and are usually degraded only slowly in mitochondria. Many polyunsaturated fatty acids (PUFA) are even inhibitors of mitochondrial β -oxidation [6].

The first indication that peroxisomes may be specifically involved in the metabolism of PUFA emerged from the demonstration of the existence of peroxisomal 2,4-dienoyl-CoA reductase in rat

liver [7]. Similarly, 3-OH-acyl-CoA epimerase appears to be a peroxisomal enzyme [8].

The third auxiliary enzyme involved in the β -oxidation of unsaturated fatty acids is Δ^3, Δ^2 -enoyl-CoA isomerase, which catalyzes the isomerization of the double bond in position 3 of acyl-CoA derivatives to position 2. As a step towards the understanding of the role of peroxisomes in the catabolism of unsaturated fatty acids, it is necessary to know whether they can metabolize double bonds at odd-numbered positions in fatty acids. This prompted us to study the subcellular distribution of this isomerase activity in the liver.

2. MATERIALS AND METHODS

The fatty acids used were all commercially available, except for Δ^3 -*trans*-dodecenoic acid, which was synthesized by condensing decyl aldehyde with malonic acid in the presence of triethanolamine [9]. The acyl-CoA esters were synthesized by the mixed anhydride method [10] and

Correspondence address: J.K.Hiltunen, Dept of Medical Biochemistry, University of Oulu, Kajaanintie 52 A, SF-90220 Oulu, Finland

purified on cellulose thin-layer plates [11]. The concentration of acyl-CoA esters was determined as described by Ellman [12] after cleaving the thioester bond with hydroxylamine at pH 7.0.

Male Sprague-Dawley rats were fed with a commercial pelleted diet supplemented with 0.3% (w/w) clofibrate for 12–14 days prior to experiments. Isolated livers were perfused with Krebs-Henseleit bicarbonate solution in equilibrium with O₂/CO₂ (19:1) without recirculation. Substrates were added by micropump-controlled infusion of stock solutions, the short-chain fatty acids in the form of sodium salts and the medium-chain-length acids in bovine serum albumin complexes. Organ spectrophotometry and surface fluorometry of the liver were performed simultaneously, as described in [13].

10% (w/v) liver homogenates were prepared in 0.25 M sucrose/0.1% (v/v) ethanol/2 mM 3-(*N*-morpholino)propanesulphonic acid (pH 7.2)/1 mM EGTA in a Potter-Elvehjem type homogenizer with a loosely fitting Teflon pestle. The homogenate was centrifuged at 800 × *g* for 10 min to separate out the nuclei (N). The heavy mitochondrial fraction (H) was obtained by centrifugation of the supernatant at 5100 × *g* for 10 min and the light mitochondrial fraction (L) was the pellet from an additional centrifugation at 27000 × *g* for 10 min. The supernatant was centrifuged at 100000 × *g* for 1 h to yield the microsomal fraction (M) as a pellet and soluble proteins as the supernatant (S) [14]. To separate the subcellular organelles further, a portion of the L-fraction from the clofibrate-fed rats was loaded onto a discontinuous sucrose gradient and centrifuged in a Sorval RC-2B centrifuge with an SS-90 vertical rotor at 40600 × *g* for 50 min as in [7].

Catalase [15], glucose-6-phosphatase [16], glutamate dehydrogenase [17] and acid phosphatase [18] were measured as described. Δ^3, Δ^2 -Enoyl-CoA isomerase was measured with 80 μ M Δ^3 -*trans*-dodecenoyl-CoA in the presence of 8 μ M rotenone [19]. Proteins were measured with the Biorad^R protein assay reagent using bovine serum albumin as a standard.

3. RESULTS

Infusion of laurate into an isolated perfused

liver resulted in an increase in the level of the catalase-H₂O₂ complex (compound I), indicating an increase in H₂O₂ production. Since peroxisomal acyl-CoA oxidase is an H₂O₂ producer, compound I also serves as a probe for the subcellular location of partial reactions of fatty acid oxidation [20,21]. Laurate infusion also resulted in some quenching of flavoprotein fluorescence, and since the latter has been shown in the liver to be mainly due to mitochondrial lipoamide dehydrogenase [22], which is in equilibrium with the mitochondrial free NADH/NAD⁺ pool, the result indicates that mitochondria are also involved in the metabolism of laurate. The increment in the level of compound I during 3-*trans*-dodecenoate infusion suggests the presence of Δ^3, Δ^2 -enoyl-CoA isomerase in peroxisomes, as isomerization of the double bond and chain shortening by one acetyl unit are required before H₂O₂ generation. The short-chain fatty acids tested here were all metabolised in mitochondria, as indicated by the quenching of flavoprotein fluorescence, but only hexanoate caused a small increase in the compound I concentration. The response pattern of the compound I level and flavoprotein fluorescence to ethanol, methanol and glycolate corroborates the proper functioning of the compartment-specific metabolic probes used (fig.1).

The enzyme activities measured in the liver homogenates are given in table 1. In order to study the subcellular localization of isomerase, the liver homogenates from the clofibrate-treated rats were fractionated by differential centrifugation. A graphic presentation of the results is given in fig.2. Recovery of enzyme activity in the fractions varied in the range 60–118% in different preparations. The distribution of isomerase resembled most that of glutamate dehydrogenase, suggesting a mitochondrial location for the bulk of the isomerase. The results of differential centrifugation of livers from control rats gave a similar distribution (not shown).

Further isopycnic centrifugation of the L-fraction on a sucrose gradient revealed a dual distribution of Δ^3, Δ^2 -enoyl-CoA isomerase activity (fig.3), the isomerase peak at a density of 45–50% sucrose coinciding with the maximum for catalase, a marker enzyme for peroxisomes, and the second, at a density of 38–42% sucrose, with glutamate dehydrogenase. Although the maximum for acid

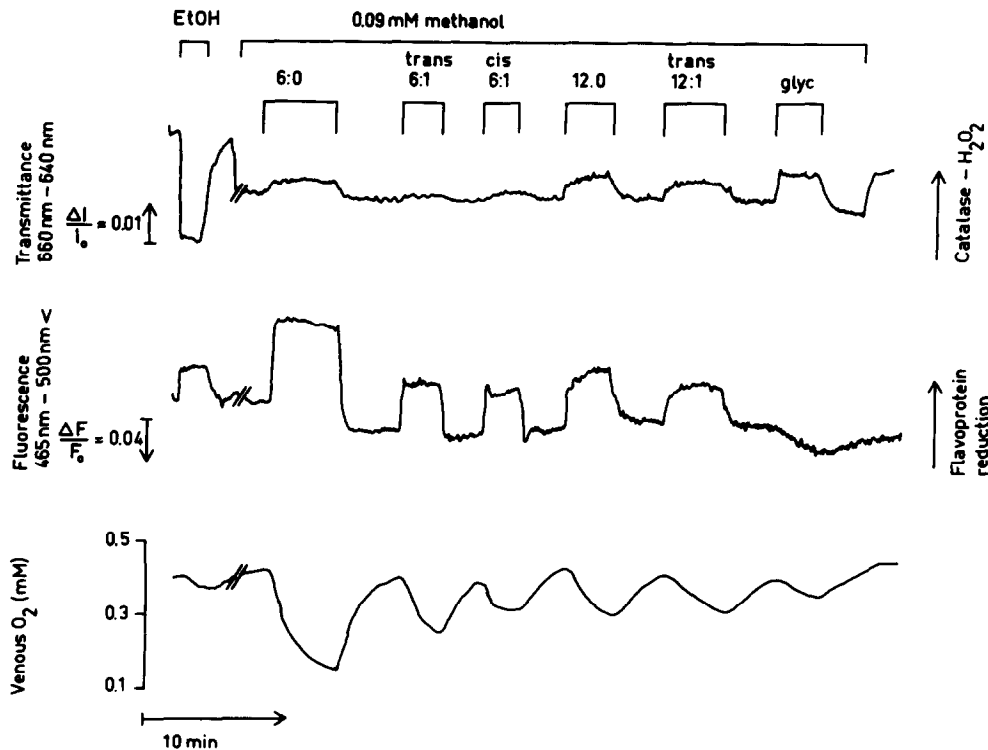


Fig.1. Catalase compound I, flavoprotein fluorescence and oxygen consumption during infusion of isolated rat liver with selected fatty acids. The perfusion rate was 4.8 ml/min per g wet wt tissue. Ethanol (EtOH) (25 mM) and methanol (0.08 mM) were present in the perfusion medium where indicated. The C₆ and C₁₂ fatty acids were infused at final concentrations of 0.71 and 0.08 mM, respectively. The glycolate (glyc) concentration was 1.0 mM. 6:0, hexanoic acid; trans 6:1, Δ^3 -trans-hexenoic acid; cis 6:1, Δ^3 -cis-hexenoic acid; 12:0, lauric acid; trans 12:1, Δ^3 -trans-dodecenoic acid.

phosphatase, a marker enzyme for lysosomes, was located in the same fraction, the data in fig.2 do not support the existence of lysosomal isomerase activity.

4. DISCUSSION

The major finding to emerge here is the direct demonstration of peroxisomal Δ^3, Δ^2 -enoyl-CoA isomerase activity, while the stimulation of H₂O₂ generation by 3-trans-dodecenoate infusion in isolated liver showed that this enzyme is also active in situ.

2,4-Dienoyl-CoA reductase is essential for the β -oxidation of fatty acids having double bonds at even-numbered carbon atoms, counted from the carboxy end of the molecule, in both mitochondria and peroxisomes [7,13,23-25]. This fact and the present demonstration of peroxisomal isomerase

Table 1
Activities of Δ^3, Δ^2 -enoyl-CoA isomerase and marker enzymes of subcellular organelles in livers from normal and clofibrate-fed rats

Component	Control	Clofibrate
Liver weight/body weight (10 ⁻³ w/w)	49 ± 2 (5)	70 ± 15 ^b (6)
Protein (mg/g liver)	199 ± 24 (6)	195 ± 32 (5)
Catalase	668 ± 81 (6)	814 ± 171 ^a (6)
Glutamate dehydrogenase	452 ± 73 (6)	509 ± 97 (6)
Δ^3, Δ^2 -Enoyl-CoA isomerase	302 ± 140 (5)	592 ± 101 ^b (4)
Glucose-6-phosphatase	140 ± 29 (6)	145 ± 42 (5)
Acid phosphatase	45 ± 8 (6)	43 ± 7 (5)

^a $P < 0.05$, ^b $P < 0.01$ (clofibrate-fed rats vs controls)

Values are means ± SD from the numbers of experiments in parentheses. Enzyme activities are given in mU/mg protein

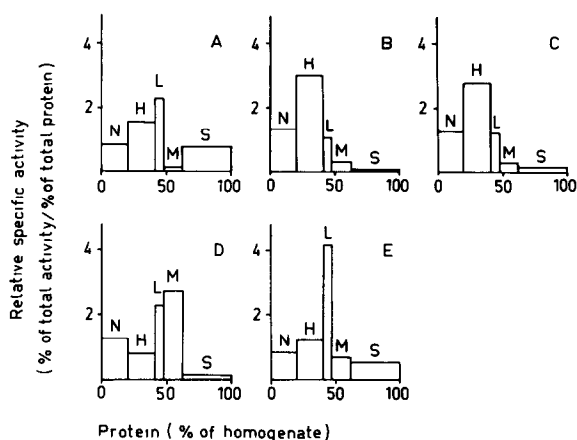


Fig.2. Separation of subcellular organelles from livers from clofibrate-fed rats by differential centrifugation. (A) Catalase, (B) glutamate dehydrogenase, (C) isomerase, (D) glucose-6-phosphatase and (E) acid phosphatase. (N) Nuclei, (H) heavy mitochondria, (L) light mitochondria, (M) microsomes, (S) soluble proteins. Results shown are means from 4–6 individual fractionation experiments.

activity together indicate that peroxisomes possess enzymes which are obligatory for the metabolism of double bonds in both even- and odd-numbered positions. Consequently, peroxisomal β -oxidation of unsaturated fatty acids should be able to pass the pre-existing double bonds. These findings are in line with one of the general physiological roles proposed for peroxisomal β -oxidation, namely prevention of the accumulation of fatty acids which are degraded only slowly in other metabolic pathways [5,6]. This conclusion is supported further by recent findings that many PUFA (excluding arachidonic acid) can undergo rapid peroxisomal β -oxidation in isolated peroxisomes, isolated liver cells and in isolated perfused rat livers [13,26].

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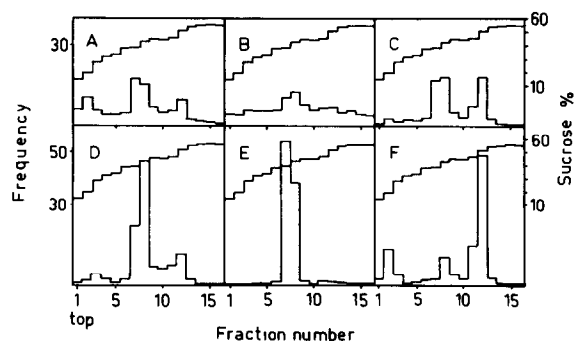


Fig.3. Subcellular localization of Δ^3, Δ^2 -enoyl-CoA isomerase rat liver as revealed by centrifugation of the L-fraction of liver homogenate in a discontinuous sucrose gradient. The upper lines in the panels give the sucrose concentrations and the lower ones the distribution of protein (A), glucose-6-phosphatase (B), isomerase (C), acid phosphatase (D), glutamate dehydrogenase (E) and catalase (F). The frequency stands for (value of parameter in fraction) \times (sum for parameter in all fractions) $^{-1} \times 100$.

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