Vol. 88, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 13, 1979 Pages 1030-1036

FATTY ACID OXIDATION BY HUMAN LIVER PEROXISOMES

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Received April 30, 1979

SUMMARY

A cyanide insensitive fatty acid oxidation system is detected in human liver and is shown to be localized in peroxisomes by subcellular fractionation in Metrizamide continuous density gradients. Fatty acyl-CoA oxidase, its characteristic enzyme, acts maximally on C_{12}-C_{18} saturated fatty acids and on oleoyl-CoA and requires FAD. These results, together with the already established properties of the system in rat liver, support its potential contribution to lipid metabolism and to the hypolipidemic effect of Clofibrate and related drugs in humans.

INTRODUCTION

The role played by liver peroxisomes in the metabolism of lipids has not been studied in humans. In rats, peroxisomes oxidize fatty acids by a cyanide insensitive system (1) which is inducible by Clofibrate (1), Nafenopin (2) and other hypolipidemic compounds (3,4) drugs also known to induce peroxisome proliferation in rat liver (3,5).

The hypolipidemic effect which these drugs also exert in humans has been attributed to an enhancement of the peroxisomal fatty acid oxidizing system (1). However this is not necessarily so, since in rats they also stimulate mitochondrial fatty acid β-oxidation (6,7) and a peroxisomal proliferative response has not been detected in liver biopsies from patients treated with Nafenopin (8). Furthermore, human and rat liver peroxisomes have a different enzymatic composition (9). In fact, to understand the role of peroxisomes in human lipid metabolism and to evaluate their contribution to the hypolipidemic action of some drugs, their presence in a fatty acid oxidizing system, and eventually its properties, must be studied.

0006-291X/79/111030-07$01.00/0

The peroxisomal fatty acid oxidizing system from rat liver catalyzes fatty acid β-oxidation by a cyanide insensitive sequence of reactions which, with the exception of the first oxidative reaction, resembles that of mitochondria (10). The α,β – desaturation of the acyl-CoA derivative, mediated in mitochondria by a dehydrogenase tightly coupled to the electron transport chain (11), is catalyzed in peroxisomes by a fatty acyl-CoA oxidase, active mainly on C_{12}-C_{18} fatty acids (2,12). This oxidase, partially characterized as a flavoprotein of 146,000 mw (13), appears as the rate limiting enzyme of the system in the liver of control rats (2) and of rats in which the system has been enhanced 7-fold by Nafenopin administration (2) or 2.4 fold by feeding a diet rich in saturated fatty acids (14).

The present report establishes the existence in human liver of a peroxisomal fatty acid oxidizing system, and describes some properties of its characteristic enzyme fatty acyl-CoA oxidase.

MATERIAL AND METHODS

Liver biopsies were obtained from four patients with normal liver function tests undergoing surgery for uncomplicated gall-stone or gastroduodenal ulcer disease. Informed consent was obtained following the procedures established by the Ethics Committee from the Medical School of the Universidad Católica de Chile. The biopsies, obtained at the beginning of surgery, were placed in ice cold 0.25 M-sucrose. Homogenization was performed in 0.25 M-sucrose containing 3 mM-imidazole HCl, pH 7.4, and 1 mM-dithiothreitol at 20-25% w/v tissue concentration. The homogenates were fractionated in continuous Metrizamide density gradients by a modification of the procedure developed by Wattiaux et al. for the isolation of lysosomes from an enriched subcellular fraction (15). 0.5 ml aliquots of the homogenate were layered on top of gradients extending from 1.10 to 1.28 g/ml in tubes from an SW-65 rotor (Beckman Instruments Inc.) and were centrifuged 90 min at 39,000 rpm, 4°C.

The activity of the peroxisomal fatty acid oxidizing system was determined from palmitoyl-CoA dependent NAD^+ reduction, as described by Lazarow & de Duve (1) with some modifications (2). The assay medium contained, at pH 8.3, 60 mM-Tris HCl, 24 μM-palmitoyl-CoA, 50 μM-CoA, 120 μM-FAD, 370 μM-NAD^+, 94 mM-nicotinamide, 2.8 mM-dithiothreitol, 2 mM-potassium cyanide, and 0.15 mg/ml of bovine serum albumin in a final volume of 0.8 ml. The enzyme containing samples were diluted to 1/2 with 1% (w/v) Triton X-100 and 5-20 μl was added to each test. Units correspond to μmol NAD^+ reduced $\cdot$ min^{-1}. g wet wt. $liver^{-1}$. The activity of the fatty acyl-CoA oxidase was determined polarographically from palmitoyl-CoA dependent oxygen consumption. The assay medium contained, at pH 8.3, 100 mM-Tris HCl,

1 mM-potassium cyanide, 200 μM-NAD^+, 31 μM-palmitoyl-CoA, 33 mM-nicotinamide, 170 μM-CoA, 50 μM-FAD, 0.6 mg/ml of bovine serum albumin, and 0.01% (w/v) Triton X-100 in a total volume of 1.6 ml. After addition of 10-50 μl of the enzyme containing sample, the basal O_2 uptake was recorder for 5 min, and the reaction started by addition of palmitoyl-CoA (2). Units correspond to μmol O_2 consumed · min^{-1}· g wet wt. $liver^{-1}$.

Established procedures were employed for the determination of marker enzymes: catalase (16) for peroxisomes, glutamate dehydrogenase (16) for mitochondria, NADPH cytochrome c reductase (17) for endoplasmic reticulum microsomes, and acid phosphatase (18) for lysosomes. Protein was measured as described by Wattiaux et al. (15) using bovine serum albumin as standard.

Crude fatty acyl-CoA oxidase preparations were made diluting the homogenates with 4 volumes of 100 mM-Tris HCl pH 8.3 and rehomogenizing in a tissue desintegrator (Virtis 45, The Virtis Co., Gardiner, New York). The supernatant obtained after centrifugation, 30 min at 100,000 g, was made 50% w/v with $(NH_4)_2$ SO_4. The precipitate, collected by centrifugation, was dissolved and dialyzed overnight in the same buffer. This preparation frees the enzyme of some components responsible for high blank readings in the assay. The final specific activity was twice that of the homogenate.

Metrizamide (2-(3-acetamido-5-N-methylacetamido-2-4-6 triiodobenzamido)-2-deoxy-D-glucose) was obtained from Nyegaard and Co., Oslo. Substrates and coenzymes were from Sigma Chemical Co., St. Louis, Missouri.

RESULTS AND DISCUSSION

In four liver biopsies studied, the activity of fatty acyl-CoA oxidase, with palmitoyl-CoA as substrate, was 0.16 ± 0.06 (S.D.) units/g liver; the activity of the peroxisomal fatty acid oxidizing system, measured as palmitoyl-CoA dependent NAD^+ reduction, was 0.24 ± 0.06 (S.D.) units/g liver. In normal rat liver, applying the same procedures, the values found were 0.81 ± 0.07 (S.D.) and 0.81 ± 0.05 (S.D.) units/g liver, respectively (2).

The subcellular distribution of the activities detected in liver homogenates, using palmitoyl-CoA as substrate, was clearly peroxisomal (Fig 1). The novel subcellular fractionation procedure developed gave good resolution of peroxisomes from mitochondria, endoplasmic reticulum microsomes and lysosomes. Approximately one half of the activity of fatty acyl-CoA oxidase and of the fatty acid oxidizing system, follows particle bound catalase. The other half, stays in the top fractions together with the rest of the catalase and of the other

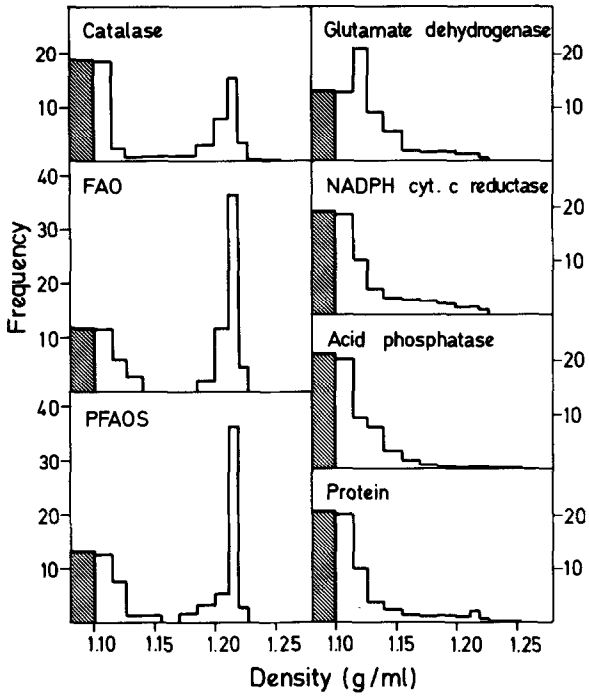

FIGURE 1

Subcellular distribution of fatty acyl-CoA oxidase (FAO) and of the peroxisomal fatty acid oxidizing system (PFAOS). Complete homogenate, from human liver, fractionated in a continuous density gradient of Metrizamide. The distribution of peroxisomes, mitochondria, endoplasmic reticulum microsomes and lysosomes was determined from marker enzymes, catalase, glutamate dehydrogenase, NADPH cytochrome c reductase and acid phosphatase, respectively. Ordinates represent average frequency of the components for each fraction, $Q/\Sigma Q\ \Delta\rho$ where Q represents the activity found in the fraction, ΣQ the total activity recovered and $\Delta\rho$ the increment in density from top to bottom of the gradient in the centrifuge tube (20). Frequency is plotted against density in a histogram form. The hatched area represents the proportion of the total activity of each component recovered in the top 0.5 ml of the tube contents which correspond to the volume of the initial homogenate layer.

marker enzymes. In separate Metrizamide gradients, designed for better resolution of the organelles equilibrating in the low density fractions, it was established that the low density fraction of catalase, fatty acyl-CoA oxidase, and the oxidizing system, follows the soluble components of the homogenate. In rat liver, a large soluble fraction of peroxisomal enzymes has also been observed and has been attributed to enzyme release from the organelles during fractionation

(5,19). Whatever the mechanism responsible for peroxisomal enzymes release might be, it affects catalase more than fatty acyl-CoA oxidase. It was concluded that fatty acyl-CoA oxidase and the fatty acid oxidizing system have a peroxisomal pattern of subcellular distribution, with little or no activity at all in mitochondria.

Human fatty acyl-CoA oxidase was further characterized employing a crude preparation obtained as described in the Materials and Methods section. This preparation gave linear assay kinetics and a 3.5-fold increase in activity upon addition of FAD (Fig 2). The effect of FAD, together with our observation establishing that the oxidase from rat liver is a flavoprotein with FAD weakly bound, suggests that human and rat liver fatty acyl-CoA oxidases are similar flavoproteins (13).

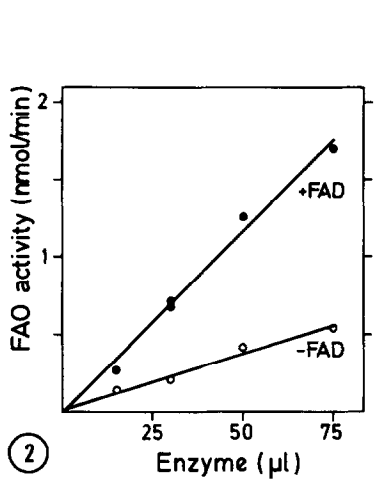

FIGURE 2

Effect of 50 µM-FAD on the rate of palmitoyl-CoA dependent oxygen consumption by a crude preparation of peroxisomal fatty acyl-CoA oxidase from human liver. The assays were performed as described in the Materials and Methods section, omitting NAD^+ and nicotinamide.

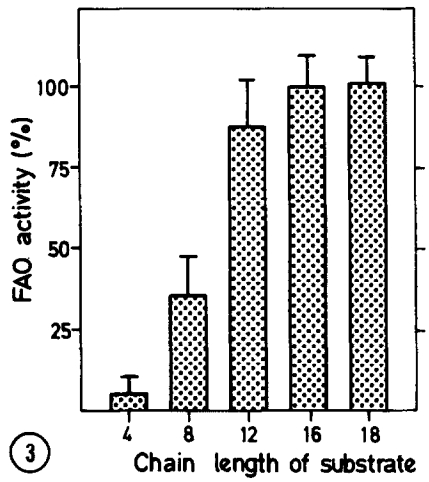

FIGURE 3

Substrate specificity of human liver oxidase with saturated fatty acids. The rate of acyl-CoA dependent oxygen consumption was measured as described in the Materials and Methods section, omitting NAD^+ and nicotinamide. A crude preparation of the enzyme was employed. Bars represent mean ± S.D. of three independent determinations expressed as percent of the activity observed with palmitoyl-CoA.

The substrate specificity of fatty acyl-CoA oxidase was checked with three different crude preparations. Maximal activities for saturated fatty acids were observed with C_{12}-C_{18} (Fig 3), as already described for the crude rat liver enzyme (2,12). With oleoyl-CoA the human enzyme gave, in each preparation, specific activities that were 90-100% of the activities observed with palmitoyl-CoA. In contrast, in rat liver the activity with oleoyl-CoA amounts to 40% of that observed with palmitoyl-CoA (2). These findings suggest that human liver peroxisomes are involved in the metabolism of saturated, as well as unsaturated fatty acids. The relative contribution of mitochondria and peroxisomes to fatty acid metabolism remains to be established. In rats, it has been suggested that the total contribution of peroxisomes to palmitate oxidation is similar to that of mitochondria (21).

In conclusion, the present work establishes the presence in human liver of fatty acyl-CoA oxidase and of the peroxisomal fatty acid oxidizing system, and supports the possibility that lipid metabolism in humans could be controlled pharmacologically and physiologically by changes induced in the capacity of peroxisomes to oxidize fatty acids.

ACKNOWLEDGEMENTS

This work was supported by the Fondo de Investigaciones de la Universidad Católica de Chile, proyecto 212/77, and by funds from the American States Organization. We thank Drs. F. Nervi for the clinical monitoring of the human volunteers, and R. Wattiaux for Metrizamide and a pre-publication copy of his work. The skilful participation of Cecilia Necochea and Maria Nelly Morales is gratefully acknowledged.

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