

# The involvement of fatty acid binding protein in peroxisomal fatty acid oxidation

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Rat liver fatty acid-binding protein (FABP) can function as a fatty acid donor protein for both peroxisomal and mitochondrial fatty acid oxidation, since <sup>14</sup>C-labeled palmitic acid bound to FABP is oxidized by both organelles. FABP is, however, not detected in peroxisomes and mitochondria of rat liver by ELISA. Acyl-CoA oxidase activity of isolated peroxisomes was not changed by addition of FABP or flavaspidic acid, an inhibitor of fatty acid binding to FABP, nor by disruption of the peroxisomal membranes. These data indicate that FABP may transfer fatty acids to peroxisomes, but is not involved in the transport of acyl-CoA through the peroxisomal membrane.

Peroxisome; Fatty acid-binding protein; Acyl-CoA translocation;  $\beta$ -Oxidation; Acyl-CoA oxidase; Rat liver

## 1. INTRODUCTION

Fatty acid-binding protein (FABP) is considered to participate in uptake and intracellular transfer of fatty acids and possibly in lipid metabolism [1,2]. The enzymes of the peroxisomal  $\beta$ -oxidation are located inside the organelle [3,4]. Fatty acids must therefore pass the peroxisomal membrane after activation by acyl-CoA synthetase or ligase, the catalytic site of which faces the cytoplasm [5]. In contrast to mitochondria, carnitine is not involved in the translocation of acyl-CoA [5–8], although short-chain and medium-chain carnitine acyltransferases are present in liver peroxisomes [9,10]. Addition of ATP to isolated peroxisomes stimulated the fatty acid oxidation; this effect was lost after disruption of the peroxisomal membranes [11]. The decrease of  $\beta$ -oxidation by treatment of peroxisomes with proteases, without disruption of the membrane, indicated the existence of a carrier protein of acyl-CoA on the cytoplasmic side of the membrane [6]. It was suggested that this protein could be FABP, since inhibitors of fatty acid binding to FABP, flavaspidic acid and bromopalmitic acid, inhibited peroxisomal  $\beta$ -oxidation and the binding of palmitoyl-CoA to isolated peroxisomes [6]. FABP can act as a fatty acid donor for rat liver and heart mitochondria [2,12], but is not present in these mitochondria [13–15]. We reinvestigated therefore the presence of FABP in peroxisomes and its role in peroxisomal fatty acid oxidation.

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## 2. MATERIALS AND METHODS

Male Wistar rats (200 g) were given daily 50 mg clofibrate in 1 ml of 60% glycerol (orally) during 10 days. After 18 h of starvation, the animals were killed by cervical dislocation. The liver was removed and immediately cooled in ice-cold buffer. Organelles were isolated from homogenized liver by differential centrifugation and centrifugation on a 28% Percoll-gradient as described [16]. Assays of urate oxidase, citrate synthase and glucose-6-phosphatase as marker enzymes for peroxisomes, mitochondria and microsomes, respectively, were according to [16].

Purification of rat liver FABP and preparation of antiserum and IgG fraction against FABP were previously described [14,17]. The FABP content of the mitochondrial and peroxisomal preparations was determined by ELISA [14], using calibration curves containing a similar content of Percoll as the organelle fraction. Recovery of FABP was 105  $\pm$  10%. [<sup>14</sup>C]Oleic acid (Amersham International, Little Chalfont, Amersham, UK) was bound to albumin in the molar ratios of 3:1 and 1:1 (spec. act. 13 000 dpm per nmol). After binding oleic acid to FABP, remaining free oleic acid was removed by incubation with Lipidex 1000 (United Technologies Packard, Downers Grove, IL, USA) at 0°C and centrifugation for 10 min at 10 000  $\times$  g (spec. act. 23 000 dpm per nmol). Substrates were incubated with the organelle fractions (0.40 mg and 0.25 mg protein for mitochondrial and peroxisomal preparations, respectively) for 2 min at 37°C in a total volume of 0.5 ml buffer (pH 7.4), containing 0.5 mM malate/0.1 mM coenzyme A/5 mM ATP/0.5 mM L-carnitine. The oxidation rates were determined from the <sup>14</sup>C-labeled acid-soluble products [18]. Acyl-CoA oxidase activity was assayed by determination of H<sub>2</sub>O<sub>2</sub>-dependent oxidation of leuko-dichlorofluorescein with 30  $\mu$ M palmitoyl-CoA as substrate at 37°C [18].

## 3. RESULTS AND DISCUSSION

The specific activities of the marker enzymes in the peroxisomal and mitochondrial preparations (Table I) show that the peroxisomal preparation is contaminated with microsomes, but contains less than 12% mitochondria. FABP was not detected

Table I

Marker enzyme activities in rat liver subcellular preparations

Marker enzyme	Mitochondria	Peroxisomes
Acyl-CoA oxidase	0.9 ± 0.7	10.6 ± 7.9
Urate oxidase	2.0 ± 1.3	12.8 ± 9.9
Citrate synthase	148.1 ± 24.9	16.5 ± 7.3
Glucose-6-phosphatase	37.5 ± 3.0	311.0 ± 86.0

Activities are means ± SD of 3 experiments and are given in nmol/min per mg protein, except for glucose-6-phosphatase in  $\mu$ mol/min per mg protein

Table II

The influence of albumin and fatty acid-binding protein on the fatty acid oxidation of rat liver mitochondria and peroxisomes

System Protein	Molar ratio oleic acid/ protein	Fatty acid oxidation rate	
		nmol/min · mg protein	%
<b>Mitochondria</b>			
Albumin	3:1	6.2 ± 0.6	100
Albumin	1:1	5.4 ± 0.7	88 ± 3
FABP	1:1	2.8 ± 0.3	46 ± 3
<b>Peroxisomes</b>			
Albumin	3:1	0.6–4.1	100
Albumin	1:1	0.4–2.2	60 ± 5
FABP	1:1	0.2–1.7	40 ± 2

Values are means ± SD of 3 experiments. The range is given for the absolute peroxisomal oxidation rates. The oleic acid concentration was 20  $\mu$ M (albumin) or 8.4  $\mu$ M (FABP)

Table III

Effect of flavaspic acid and FABP on acyl-CoA oxidase activity of peroxisomes

Addition	Acyl-CoA oxidase activity (nmol H <sub>2</sub> O <sub>2</sub> /min per mg protein)	
	Peroxisomes	Frozen-thawed peroxisomes
None	4.7 ± 1.2	5.6 ± 1.4
30 $\mu$ M flavaspic acid	4.2 ± 1.2	5.0 ± 1.0
0.87 $\mu$ M FABP	4.5 ± 0.7	5.3 ± 1.2

Values are means ± SD of 3 experiments. Freezing and thawing of the peroxisomes was performed 3 times

(<0.16 nmol/mg) in the mitochondrial and peroxisomal fractions by ELISA. Cell fractionation, immunoblot analysis and immunoelectron microscopy did not show the presence of FABP in mitochondria either [13–15]. Competitive inhibition of fatty acid binding by flavaspic acid to peroxisomes does not necessarily implicate the involvement of FABP as suggested by Appelkvist and Dallner [6], since a specific fatty acid binding by the membrane lipids may take place. Mitochondrial oxidation rates of oleic acid, bound to albumin or FABP (Table II) are in the same range as

those of palmitic acid, bound to these proteins [2]. Although the absolute peroxisomal oxidation rates varied, the relative values show that oleic acid bound to FABP was oxidized to the same extent by peroxisomes as albumin-bound oleic acid. These data indicate that FABP can act as a fatty acid donor protein for peroxisomal and mitochondrial  $\beta$ -oxidation.

Addition of flavaspic acid, an inhibitor of fatty acid binding to FABP [17], or FABP had no influence on the acyl-CoA oxidase activity (Table III). Three times freezing and thawing, to disrupt the peroxisomal membrane, also had no effect. This indicates that the transport of palmitoyl-CoA was not rate-limiting for this enzyme, which is the regulatory enzyme of peroxisomal oxidation [18]. A slight increase of palmitoyl-CoA-dependent NAD<sup>+</sup> reduction was previously found after sonication or addition of Triton X-100 [6].

We conclude that FABP can function as a fatty acid donor for peroxisomal  $\beta$ -oxidation, but is not involved in the translocation of acyl-CoA esters through the peroxisomal membrane as previously suggested [6].

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