REGULATION OF PEROXISOMAL FATTY ACID OXIDATION

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1. Introduction

Isolated rat liver peroxisomes have been shown to β-oxidize fatty acids [1,2]. This activity is induced by some hypolidaemic drugs [3]. Peroxisomal β-oxidation is also induced by di-(2-ethylhexyl)phthalate [4], and by high fat diets [5]. Most measurements of β-oxidation by isolated peroxisomal fractions have been carried out in grossly hypotonic media, also incorporating Triton X-100, to achieve optimum sensitivity [1]. Although such an assay gives an excellent measure of the enzymic activities present, it is likely to give less reliable information as to how peroxisomal β-oxidation operates in vivo. Also peroxisomal fractions are usually isolated in highly hypertonic sucrose density gradients [1,6], which may irreversibly damage regulatory properties present in the intact peroxisomal particle. It is well known that liver mitochondrial β-oxidation is severely impaired even by isotonic sucrose media [7].

We have therefore studied the properties of peroxisomal fractions isolated in an isotonic Percoll gradient. Fatty acid oxidation studies were subsequently carried out in an isotonic KCl containing incubation medium. These studies suggest that the access of substrate (acyl-CoA ester) to the peroxisomal β-oxidation system is regulated.

2. Materials and methods

Palmitic acid and erucic acid ($C_{22:1(9)}$) were obtained from Fluka AG, Buchs. Gadoleic acid ($C_{20:1(11)}$) was obtained from Nu-Chek Preps., Elysian, MN. [U-^{14}C]palmitic acid was purchased from the Radiochemical Centre, Amersham, and [14-^{14}C]erucic acid from CEA, Gif-sur-Yvette. [U-^{14}C]palmitoyl-CoA and [14-^{14}C]erucoyl-CoA and unlabelled acyl-CoA esters were synthesized and characterized as in [8]. Coenzyme A (grade 1-L) was obtained from Sigma Chemical Co., St Louis, MO. Percoll was purchased from Pharmacia Fine Chemicals AB, Uppsala. All other reagents were of analytical grade, or of highest purity available.

Liver peroxisomes were prepared from male albino Wistar Rats (200–250 g) which had been fed a standard pelleted diet containing 0.3% (w/w) clofibrate for 10–14 days. A subcellular fraction 'L' was prepared as in [6]. About 1 ml (corresponding to ~100 mg protein) was layered on the top of 29 ml 50% (v/v) Percoll, containing 250 mM sucrose, 2 mM Mops (4-morpholinepropane-sulphonic acid), 1 mM EGTA, and 0.1% (v/v) ethanol (pH 7.2). The gradient was formed, and the fractionation carried out, during a 30 min centrifugation at 63 000 × g_{av} in the TV-850 rotor. The Sorvall OTD-65 Ultracentrifuge was used. The pooled peak peroxisomal fractions were diluted with 3 vol. 250 mM sucrose, containing 10 mM Mops, 1 mM EGTA, (pH 7.2). The diluted suspension was centrifuged at 32 000 × g_{av} for 15 min and the supernatant removed by aspiration. The sediment was resuspended in remaining medium, giving final conc. 20-30 mg protein/ml. This suspension was used in all studies of fatty acid oxidation, usually within 3–4 h of the animals being killed. This fraction contained 13% of the uricase (EC 1.7.3.3) activity present in the starting homogenate. The cytochrome *c* oxidase (EC 1.9.3.1) activity was 0.6% of that in the starting homogenate. Contamination by endoplasmic reticulum was similarly ~0.5% (measured as rotenone-insensitive NADPH cytochrome *c* reductase (EC 1.6.2.3), and ~1.4% with respect to lyzosomes (measured as

β-*N*-acetyl-D-glucoseaminidase activity EC 3.2.1.29).

Peroxisomal fatty acid oxidation was measured spectrometrically as acyl-CoA dependent NAD reduction in an assay medium containing 130 mM KCl, 10 mM Hepes, 1 mM EGTA, 1–2 mg defatted bovine serum albumin/ml, 0.2 mM NAD (pH 7.2). CoA was added as indicated. Rates of NAD reduction were measured by using an Aminco DW-2 spectrophotometer operated in dual wavelength mode (340–400 nm).

Peroxisomal oxidation of labelled acyl-CoA esters was measured as release of acid-soluble (5%, v/v, $HClO_4$) radioactivity. The incubation medium used was similar to that used in the spectrometric assay, except that bovine serum albumin was included to 2 mg/ml and NAD to 0.5 mM because of the much longer incubation times. Antimycin A (5 μg/ml) was also included in the assay to block contaminating mitochondrial β-oxidation. All assays were carried out at 37°C.

Solutions of acyl-CoA esters were assayed enzymatically as in [8]. Proteins were measured by using the Bio-Rad protein assay kit (Bio-Rad Labs).

3. Results and Discussion

We have shown that peroxisomal β-oxidation seems particularly well suited for the oxidation of long monounsaturated fatty acids, in contrast to saturated fatty acids of similar chain lengths [8]. From these data it is also apparent that the relative rates of peroxisomal β-oxidation of erucoyl-CoA (compared to that obtained with palmitoyl-CoA) is similar to that of mitochondrial β-oxidation, i.e., ~45–50% of the rate given by palmitate. This contrasts the marked increase in oxidation of erucic acid observed with hepatocytes isolated from clofibrate-treated animals. Although the hepatocyte oxidation of palmitate is doubled on clofibrate treatment, a 5-fold increase in oxidation of erucic acid was observed [9]. It is therefore possible that there is a mechanism by which the type of fatty acids which are subjected to peroxisomal β-oxidation is regulated.

The results presented in fig. 1 show that peroxisomal

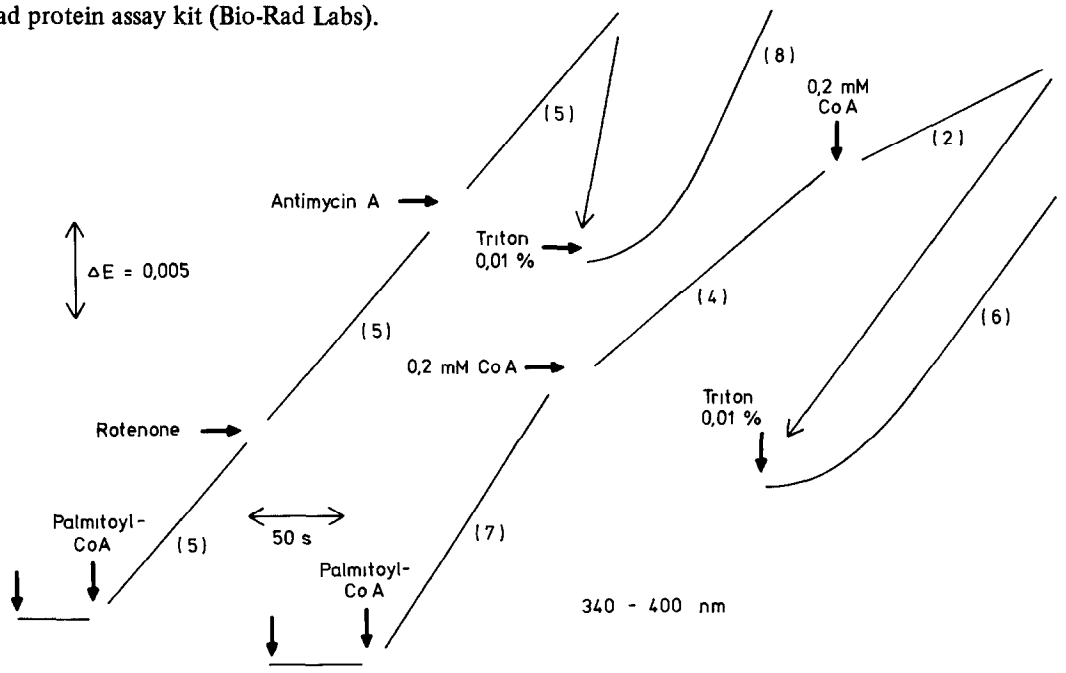

Fig. 1. Effect of respiratory inhibitors, CoA and Triton X-100 on peroxisomal oxidation of palmitoyl-CoA. Typical traces show palmitoyl-CoA dependent NAD reduction obtained as in section 2. The point of addition of rotenone (6 μg), antimycin A (6 μg), CoA and Triton X-100 have been indicated by arrows. Additions of 0.2 mM NAD and 0.1 mM CoA have been indicated by an unlabelled arrow. Each assay contained 260 μg peroxisomal protein and 1.2 mg defatted bovine serum albumin and 50 μM palmitoyl-CoA in 1 ml total assay volume. The numbers in parenthesis represent rates of NAD reduction expressed as nmol NADH formed .min^{-1} .mg peroxisomal $protein^{-1}$.

β-oxidation, as measured by the spectrophotometric assay, is insensitive to both antimycin A and rotenone, both of which are powerful inhibitors of mitochondrial fatty acid oxidation. Also no CoA need be added to achieve a linear steady rate. With the hypotonic, solubilised, assay [1] only a small burst of activity is achieved in the absence of added CoA [1]. With an isotonic assay, the addition of CoA is inhibitory to palmitoyl-CoA oxidation (see fig. 1). Successive additions of 0.2 mM CoA decreases the rate of oxidation to ~30% of the starting rate (see fig. 1). The subsequent addition of 0.01% Triton X-100, leads to an increased oxidative rate, which is not less than that observed in the absence of added CoA (fig. 1). When Triton X-100 is added prior to CoA no inhibition by CoA is observed (see fig. 2). The final rate of oxidation achieved when Triton X-100 is added prior to CoA and palmitoyl-CoA is very similar to that obtained with the hypotonic, solubilised assay (20 mM P_i, 0.2 mM NAD, 0.1 mM NAD and 0.1 mM CoA, pH 7.4) (see fig. 2).

These findings show that palmitoyl-CoA does not have unhindered access to the β-oxidation system, and that this access can be modulated by CoA. Increasing concentrations of CoA inhibits the access of substrate to the β-oxidation system. The observation that steady linear rates are achieved in the absence of added CoA also suggests that the peroxisomal matrix contains a CoA pool, and that added CoA may not readily penetrate the peroxisomal membrane. This is markedly different from the strict requirement for added NAD (app. K_m ~25 μM). The site of access modulation cannot be ascertained from these studies, but the effects of Triton X-100 strongly suggest that the peroxisomal membrane is involved.

Results shown in fig. 3 suggest that the inhibitory

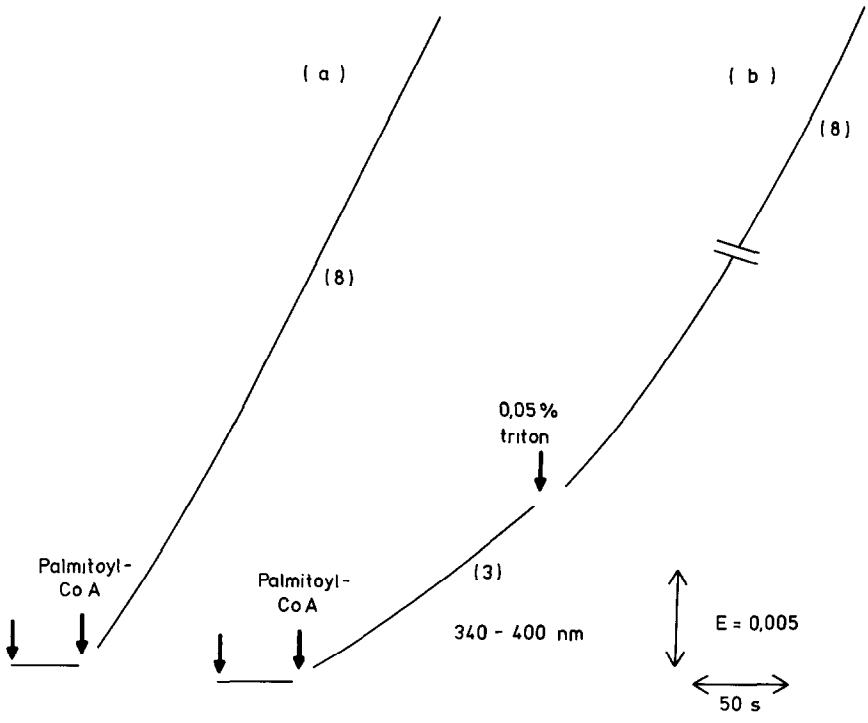

Fig. 2. The removal by Triton X-100 of inhibition of palmitoyl-CoA oxidation by added CoA. Typical recorded traces showing palmitoyl-CoA (25 μM)-dependent reduction of NAD (a) in a hypotonic assay medium (20 mM P_i, 0.5 mM dithiothreitol and 0.001% Triton X-100, pH 7.4) and (b) in the isotonic KCl medium in section 2. Addition of 0.2 mM NAD and 0.1 mM CoA is indicated by unlabelled arrows. Other additions are indicated in the figure. The assays contained 1.2 mg defatted bovine serum albumin and 240 μg peroxisomal protein, in 1 ml final assay volume. Additions of 0.2 mM NAD and 0.1 mM CoA are indicated by unlabelled arrows. The numbers in parentheses represent rates of NAD reduction expressed as nmol NADH formed . min^{-1} . mg peroxisomal $protein^{-1}$.

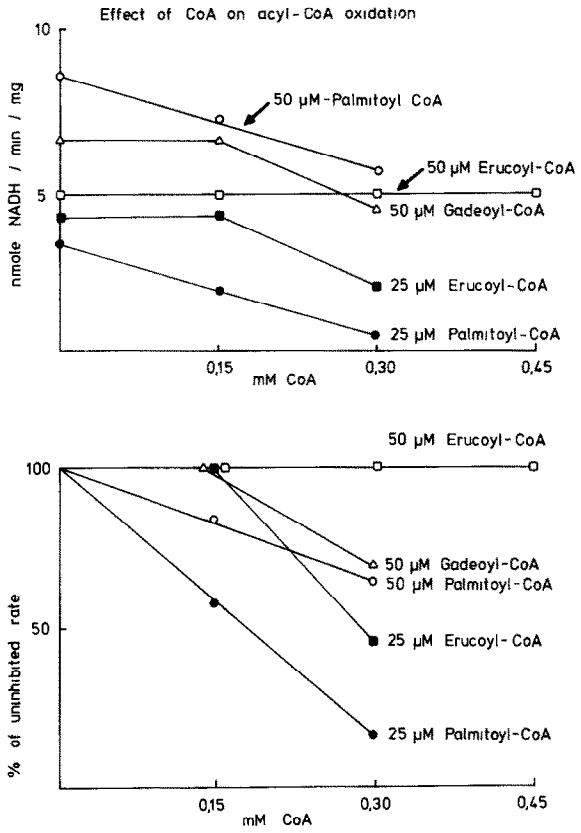

Fig. 3. Effect of various CoA concentrations on oxidation of acyl-CoA esters of different chain lengths. Rates of acyl-CoA-dependent NAD reduction was measured spectrophotometrically as in section 2. Measured rates of NAD reduction have been plotted (a) against the concentration of subsequently added CoA, and (b) as % inhibition of initial rates of oxidation obtained in the absence of added CoA. Acyl-CoA esters were added as indicated in the figure. Each assay also contained 1 mg defatted bovine serum albumin, in 1 ml final assay volume. Each assay contained 180 μg peroxisomal protein.

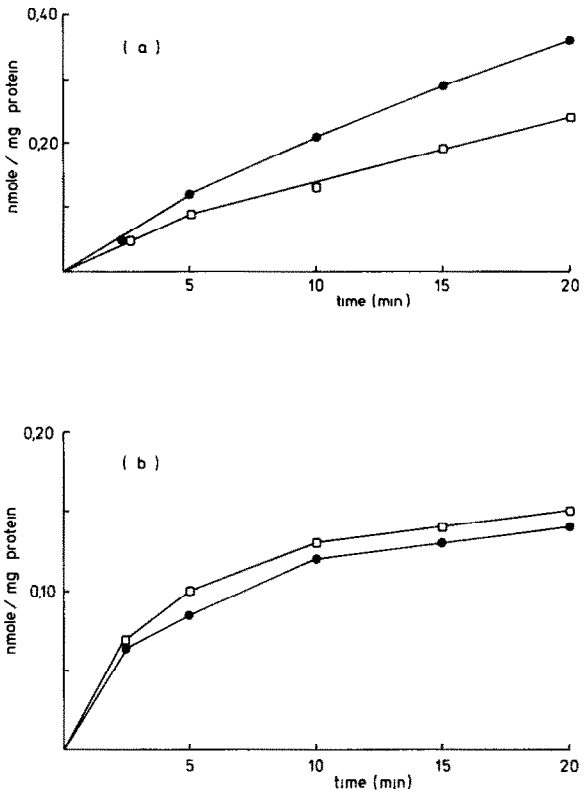

Fig. 4. Effect of added CoA on peroxisomal oxidation of [U-^{14}C]palmitoyl-CoA and [14-^{14}C]erucoyl-CoA. The oxidation of (a) [U-^{14}C]palmitoyl-CoA and (b) [14-^{14}C]erucoyl-CoA was measured as acid-soluble radioactivity released after various times of incubation, as in section 2. The oxidation of these acyl-CoA esters was followed in the absence of added CoA (•) and in the presence of added 0.3 mM CoA (□). Both [U-^{14}C]palmitoyl-CoA (spec. act. 480 dpm/nmol) and [14-^{14}C]erucoyl-CoA (spec. act. 8100 dpm/nmol) were included in the assay to 50 μM final conc. Each assay contained 2.8 mg peroxisomal protein in 1.4 ml total incubation volume.

effect of CoA on peroxisomal β-oxidation is dependent on the chain length of the acyl-CoA ester. The oxidation of erucoyl-CoA is unaffected by added CoA up to 0.45 mM. Similarly, the oxidation of gadeoyl-CoA is less inhibited by added CoA than is the oxidation of palmitoyl-CoA. Figure 4 shows that added 0.3 mM CoA inhibits the oxidation of [U-^{14}C]palmitoyl-CoA by > 30%. There is no effect, or probably a small stimulation, by the same concentration of CoA on the oxidation of [14-^{14}C]erucoyl-CoA.

These findings suggest that the concentration of free CoA can modulate both the type of fatty acids which are oxidized by peroxisomes through a chain length selective control of their rate of oxidation. Such a mechanism would facilitate peroxisomal oxidation of very long chain fatty acids, while shorter fatty acids are funnelled to mitochondrial β-oxidation. Is it likely that this mechanism could operate in vivo? If it does operate in the intact hepatocyte it could explain the differences described regarding clofibrate-poten-

tiated oxidation of palmitate and erucate by isolated hepatocytes [9]. Also the range of CoA concentrations which are effective in vitro are not dramatically different from estimates of the cytosolic CoA concentration (~0.1 mM [10]). It is also possible that there may be local variations in CoA concentrations in the cytosolic space.

It is also significant in this context that peroxisomal fatty acid oxidation is induced following feeding of a high fat diet, particularly when the fat contains a high fraction of very long chain fatty acids, e.g., partially hydrogenated marine oils [5]. On the basis of the findings presented here peroxisomal β-oxidation would be expected to be optimally active when most of the cytosolic CoA is acylated. As the cytosolic CoA concentration is increased peroxisomal β-oxidation would preferentially oxidize very long chain fatty acids to shorter chain length products. Peroxisomal β-oxidation therefore acquires the character of an auxilliary β-oxidation system which is most active when the liver is required to metabolise a sustained high influx of fatty acids, and in particular when these are less desirable as substrates for mitochondrial β-oxidation, e.g., $C_{22:1}$ fatty acids.

Acknowledgements

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