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COMPARISON OF FATTY ACID OXIDATION IN MITOCHONDRIA AND PEROXISOMES FROM RAT LIVER*

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SUMMARY

Oxygen uptake with succinate or palmitoyl-CoA as substrates can be measured in rat liver mitochondria that have been isolated by sucrose density gradient centrifugation providing the fractions are diluted with a 30 mM phosphate buffer rather than with an isotonic medium. Separate assay procedures were used to measure peroxisomal and mitochondrial β-oxidation of palmitoyl-CoA in the fractions of a sucrose gradient used to separate these organelles. A preliminary estimate of the ratio of palmitoyl-CoA oxidation by the mitochondrial fraction relative to the surviving peroxisomes from livers of male rats was 3.2.

INTRODUCTION

Lazarow and de Duve (1,2) reported fatty-acid β-oxidation in peroxisomes isolated by sucrose gradients from rat liver. The bulk of the palmitoyl-CoA oxidation detected on the gradient occurred in the peroxisomes and not in the mitochondria. We have confirmed their results but were concerned by the lack of β-oxidation activity in the mitochondrial fraction where this system has traditionally been reported to be located. During sucrose density gradient centrifugation, mitochondria are damaged to the extent that the rates of respiration are very low with succinate, palmitoyl-CoA, or malate as substrates in an isotonic assay medium. However, we have been able to demonstrate ADP-stimulated respiration as 0_2 uptake by mitochondria from a sucrose density gradient by using dilute phosphate buffer as the assay medium. This enabled us to assay β-oxidation polarographically across the gradient to determine its distribution between the mitochondria and peroxisomes.

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MATERIALS AND METHODS

Subcellular organelles from the livers of male, Sprague-Dawley rats that had been starved the previous night were separated by isopycnic sucrose density gradient centrifugation (3,4). The buffer for both the grinding medium of 0.25 M sucrose and the sucrose gradient was 1 mM sodium phosphate at pH 7.5. Marker enzyme for peroxisomes was catalase (3), and for mitochondria, glutamate dehydrogenase (5) with Triton X-100 included in both assays. Protein was determined by a modified Lowry procedure (6), and sucrose by refractometry.

The peroxisomal assay for β-oxidation was measured by the rate of oxygen uptake in a medium of NAD, coenzyme A, Triton X-100, bovine serium albumin and dithiothreitol as used by Lazarow and de Duve (1) except that 0.22 μg of antimycin A in 20 μl of ethanol was added per ml of reaction mixture in place of KCN. Reactions were run at 35^{o} using a Clark type oxygen electrode (Rank Brothers, Bottisham, Cambridge, England), and the oxygen concentration of air saturated distilled water at 35^{o} was taken to be 231 μM (7).

Mitochondrial respiration was measured as oxygen uptake similarly to peroxisomal respiration but in two different assay media. Aliquots of 200 μl from gradient fractions or the pooled mitochondrial fractions were diluted into an isotonic medium described by Bieber et al. (8). For the other assay, samples were diluted five fold in the assay chamber by adding them to a buffer of 30 mM potassium phosphate at pH 7.5 and 100 μM ADP, so that the final sucrose concentration was 0.36 to 0.40 M. After a 2 min incubation period, an endogenous rate of oxygen uptake with ADP was measured for 2 min and then the reaction was initiated with a substrate. The endogenous rate with ADP was substrated from the rate after the addition of the substrate. The final substrate concentrations were either 10 μM palmitoyl-CoA, 10 mM malate, 10 mM succinate, or 100 μM NADH.

RESULTS

The subcellular organelle distribution in an isopycnic sucrose density gradient was characterized by using catalase activity for peroxisomal location and glutamate dehydrogenase activity for mitochondria (Fig. 1, top). The one peak of palmitoyl-CoA dependent oxygen uptake using Lazarow and de Duve's (1) assay procedure was the same as the peak for peroxisomal catalase (Fig. 1, bottom). No activity was found in the mitochondrial fractions with these assays. These results confirmed those of Lazarow and de Duve (1), but did not show the distribution of β-oxidation between the mitochondria and peroxisomes.

The rates of mitochondrial respiration after dilution of fractions into the isotonic buffer (8) with ADP and substrates of succinate, NADH, or palmitoyl-CoA were very low (Table 1). When diluted into the phosphate buffer as the assay medium, rates were much higher than those with the isotonic buffer. The oxygen uptake with all three substrates was cyanide sensitive. The oxidation of both succinate and palmitoyl-CoA was antimycin A sensitive. The NADH oxidation was not sensitive to antimycin A or rotenone but was stimulated by added cytochrome c and inhibited by cyanide. Therefore, the electrons

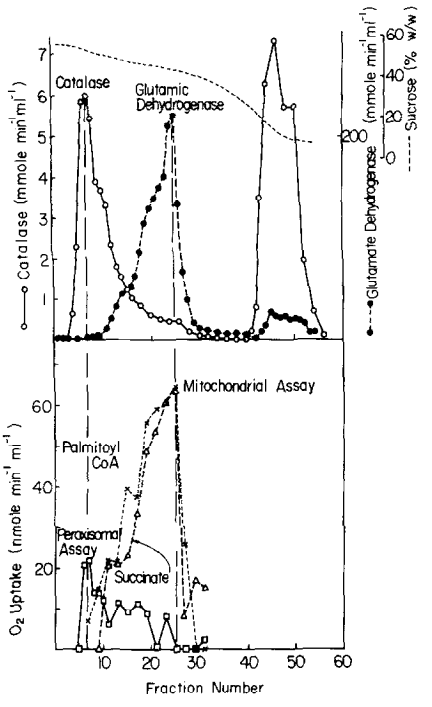

FIGURE 1

Distribution of Palmitoyl-CoA Oxidation Between Peroxisomes and Mitochondria from a Sucrose Density Gradient. The gradient is characterized at the top of the figure by the sucrose concentration (- - -) and by the distribution of catalase (0——0) and glutamate dehydrogenase (●- - -●) which are markers for peroxisomes and mitochondria, respectively. In the lower part of the figure, peroxisomal β-oxidation with palmitoyl-CoA as the substrate (□——□) was measured by oxygen uptake in the medium used by Lazarow and de Duve (1) and mitochondrial respiration with palmitoyl-CoA (x- - -x) or succinate (Δ- - - -Δ) was measured after dilution into a phosphate assay medium. The rates are expressed per ml of sample.

from NADH probably did not pass through most of the electron transport chain, but rather through the NADH-cytochrome c reductase of the outer membrane. The rate of oxygen uptake using malate as the substrate was low in both buffers. It appears that these mitochondria only use substrates that donate their electrons after site 1 or that NADH dehydrogenase was inactive.

When the gradient fractions were assayed after dilution into 30 mM phosphate buffer, β-oxidation could be measured in the mitochondrial fractions (Fig. 1). The rates of ADP stimulated succinate and palmitoyl-CoA oxidation peaked in the same fraction as the glutamate dehydrogenase mitochondrial marker.

The area under the curves for oxygen uptake using palmitoyl-CoA in the peroxisomal and mitochondrial regions of the gradient should indicate the relative capacities of the organelles for β-oxidation. In the peroxisomes, only one atom of oxygen is taken up during fatty acyl CoA oxidation, as the reduced NAD from the oxidation of the unsaturated fatty acyl CoA is not thought to be oxidized by the isolated particles (9). In mitochondria, assuming that all of the NADH produced during β-oxidation is oxidized, two atoms of 0_2 would be taken up per acetyl CoA released. Therefore, the area under the mitochondrial palmitoyl-CoA oxidation curve should be divided by two for comparison with the amount of peroxisomal oxidation. Based on these tentative considerations, the amount of palmitoyl-CoA oxidation in the total mitochondrial fraction was 3.2 times that in the surviving peroxisomal fraction from the gradient.

DISCUSSION

We found little or no 0_2 uptake by rat liver mitochondria isolated by sucrose density gradients with succinate, palmitoyl-CoA, NADH, or malate as substrates in an isotonic assay medium. Douce et al. (10) have reported that plant mitochondria purified in sucrose gradients actively take up 0_2 in an isotonic assay medium with appropriate substrates, but that rat liver mitochondria do not. However, we could observe mitochondrial respiration by diluting the mitochondria from the sucrose gradient into 30 mM potassium phosphate at pH 7.5 to a final concentration of 0.36 to 0.40 M sucrose. Presumably, the mitochondria from the gradient needed to be swollen in the dilute buffer before they would respire. Electron microscopic examination of the mitochondria from the gradient before dilution showed them to be mainly in the condensed form. Although these liver mitochondria from the sucrose gradient respired upon dilution in phosphate buffer, the rates were stimulated only 10% by ADP and they did not cycle between states 3 and 4 as do intact mitochondria. Therefore these mitochondria were mostly uncoupled.

We used enough antimycin A in the fatty acid β-oxidation assay used by Lazarow and de Duve (1) to completely inhibit the oxidation of succinate and palmitoyl-CoA in the peak mitochondrial fraction (Table 1). Therefore that assay detected only the peroxisomal β-oxidation. The assay without antimycin A used for mitochondria palmitoyl-CoA oxidation was stimulated by ADP and sensitive to cyanide and antimycin A and should

TABLE I

Oxygen Uptake By Mitochondria. The fractions containing mitochondria from a sucrose gradient similar to that in Figure 1 were pooled and 200 μl samples containing 0.48 mg of protein were used in each assay. Where indicated, 0.22 μg of antimycin A per ml was included in the assay. Endogenous rates of 2 nmol min^{-1} mg $protein^{-1}$ in the phosphate medium and 7 nmol min^{-1} mg $protein^{-1}$ in the isotonic medium were substrated from the rates in the presence of substrate to yield these net rates.

	Assay Medium		
	30 mM Phosphate		Isotonic
Substrate	- antimycin A	+ antimycin A	- antimycin A
	nmol min^{-1} mg $protein^{-1}$		
Succinate	35	1	0
Palmitoyl-CoA	20	2	5
NADH	6	-----	0
NADH + 20 μM cytochrome c	67	70	-----
Malate	4	-----	0

have been specific for the mitochondrial system. Activity in this medium due to peroxisomes in the lower portion of the gradient should have been very low since the NAD, coenzyme A, Triton X-100, dithiothreitol and bovine serum albumin components of the peroxisomal assay were omitted from the mitochondrial assay medium.

Possibilities for error exist in this preliminary estimate of the relative capacities of peroxisomes and mitochondria for palmitoyl-CoA oxidation. The conditions of the assay for mitochondrial β-oxidation may not be optimal, thereby underestimating the activity in the mitochondria. Breakage of peroxisomes may cause us to underestimate the amount of peroxisomal β-oxidation. There was a large amount of peroxisome breakage as evidenced by the amount of catalase recovered at the top of the gradient. Very little peroxisomal β-oxidation activity was recovered at the top of the gradient, but the peroxisomal β-oxidation system may not be active unless compartmented in the peroxisome. If it is assumed that all of the catalase is peroxisomal, (which may not be a valid assumption

(11)), 61% of the peroxisomes were broken. This factor would change the ratio of mitochondrial to peroxisomal palmitoyl-CoA oxidation to 1.2.

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