ELONGATION OF PALMITYL-CoA IN MOUSE BRAIN MITOCHONDRIA. COMPARISON WITH STEARYL-CoA.

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SUMMARY : In mouse brain mitochondria, palmityl-CoA is elongated by acetyl-CoA in the presence of NADH and NADPH providing hydroxy and non hydroxy fatty acids mainly saturated stearic acid. Using NADH alone increases the production of hydroxy acyl-CoA. Similar patterns of enzymatic activity and cofactor and substrate requirements are found for stearyl-CoA elongation presuming that the same enzyme system elongates both acyl-CoA (unlike in microsomes).

INTRODUCTION

Different systems are involved for brain fatty acid biosynthesis in three main cellular compartments : mitochondria, microsomes (1, 2) and cytosol (3, 4).

In mitochondria, contradictory results have been obtained in different tissues (5-15) and few studies have been made in brain. Acetyl-CoA is incorporated into endogenous fatty acids (1, 16, 17) as well as in added acyl-CoA (18, 19). We have shown (19) that a "de novo" synthesis occurs in brain as it has been demonstrated by others in liver (14, 20) using malonyl-CoA as carbon unit donor and providing mainly C_{16} (85 %) and few C_{14} and C_{18}. Adding palmityl-CoA or longer chain acyl-CoA to the incubation medium inhibits this synthesis ; but if acetyl-CoA replaces malonyl-CoA, an elongation occurs.

The purpose of this study is to determine the optimal conditions for palmityl-CoA elongation in mouse brain mitochondria and to determine the reaction products. Comparison with stearyl-CoA elongation indicates major differences between mitochondrial and microsomal systems.

MATERIAL and METHODS

Mitochondrial preparation : Mitochondrial preparation of 18 day-old mice and the purity of this fraction have been previously described (19). Briefly tissue is washed and homogenized in cold 0.32 M sucrose, 0.01 M phosphate pH 7 buffer. The homogenate is spun at 2 000 g for 5 min. Pellet containing heavy myelin fragments, plasma membranes and cellular debris is discarded and the supernatant is respun under the same conditions. Then the supernatant is centrifuged at 12 500 g for 30 min. and the resuspended pellet, free of microsomes, is layered on a discontinuous sucrose gradient (0.8 M, 1.2 M) and spun at 53 000 g for 2 hours. Myelin and synaptosomes are discarded with the supernatant and the pellet containing pure mitochondria is resuspended in 1.5 ml of sucrose-phosphate buffer. Proteins were determined by Lowry's method (21).

Assay conditions : The assay for acyl-CoA elongation was measured by determining the amount of radioactive acetyl-CoA incorporated into fatty acids. Routinely the incubation mixture involves 50 μM acetyl-CoA (2 mCi/mMole), 500 μM NADPH, 500 μM NADH, 30 μM of palmityl-CoA or stearyl-CoA, 1 mg Triton X-100, 1 mg mitochondrial proteins and sucrose-phosphate buffer up to 1 ml. After 30 min. of incubation at 37°C, the reaction is stopped by the addition of 0.5 ml of 15 % alcoolic potassium hydroxide

and saponified for 30 min. in a boiling water-bath. The reaction mixture is then acidified with 0.5 ml 5.5 N HCl and fatty acids are extracted twice with 5 ml petroleum ether. Both extracts are mixed and the solvent is evaporated to dryness. The residue is counted and possibly methylated (22).

Identification of fatty acids methyl esters is accomplished by a combination of thin-layer and gas-liquid chromatographies. Separation of hydroxy and non hydroxy methyl esters is performed on silica gel thin-layer chromatography using as solvent petroleum ether-ether 80:20 v/v. Gas-liquid chromatography is performed on a 3 % SE 30 column using a Packard 5750 apparatus. The radioactivity of the eluate is measured with a Panax counter.

When total lipids were studied by thin-layer chromatographies, the reaction was stopped by 5 ml of chloroform-methanol 2:1 v/v. Lipids were extracted twice (23, 24) and washed using the Folch partition method (25). Hydroxy and non hydroxy fatty acids were separated as previously described. Possibly non esterified fatty acids were first discarded by thin-layer chromatography using as solvent chloroform-acetic acid 90:10 v/v, other lipids remaining with the initial spot.

Each experiment was performed a minimum of three times.

RESULTS

Palmityl-CoA and stearyl-CoA are both elongated at an optimal rate when the concentration of the acyl-CoA is 40 µM, as shown on Figure 1. Over this concentration an inhibition occurs probably due to critical micellar concentration or inhibition by excess of substrate or detergent effect.

The effect of NADH and NADPH on palmityl-CoA elongation is illustrated on Figure 2. Without any nicotinamide nucleo-

Figure 1 : Incorporation of radioactive acetyl-CoA into palmityl-CoA and stearyl-CoA in the presence of varying concentration of acyl-CoA.

Palmityl-CoA : △ Stearyl-CoA : ▲

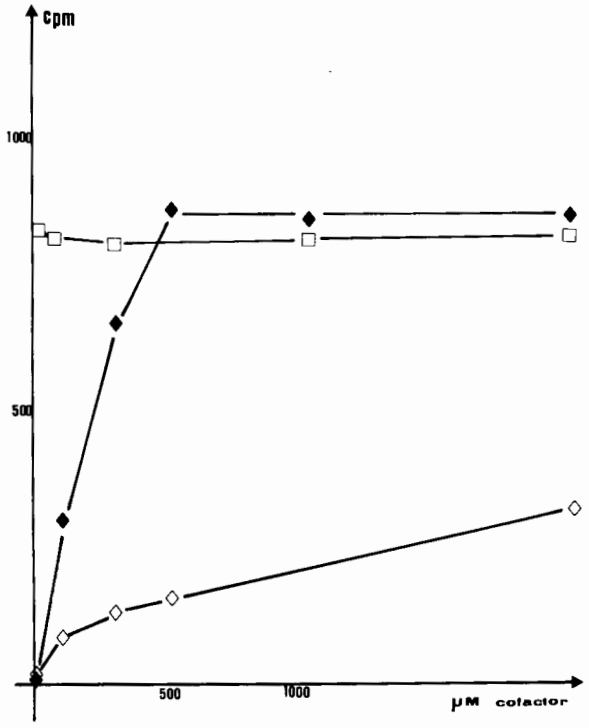

Figure 2 : Effect of NADH and NADPH on palmityl-CoA elongation by radioactive acetyl-CoA.

NADH : ◆ NADPH : ◇

NADPH in the presence of 500 µM NADH : □

tide no synthesis occurs. The best nucleotide is NADH. The optimal concentration is 500 µM. Higher values did not increase the biosynthesis. NADPH alone is less efficient ; only 1/3 of the elongation activity is reached for a concentration of 2 mM. Adding varying concentration of NADPH in the presence of the optimal concentration of NADH (500 µM) does not increase the rate of palmityl-CoA elongation.

Chromatographic analysis of the reaction products shows the same action of both nucleotides, either with palmityl-CoA or with stearyl-CoA. In the presence of NADH (500 µM) and

Table I : Thin-layer chromatographic analysis of the reaction products of palmityl-CoA elongation by acetyl-CoA.

		H.F.A.	N.H.F.A.
L.F.A. + N.E.F.A. %	NADH	60 ± 6	35 ± 4
	NADH + NADPH	25 ± 3	72 ± 7
L.F.A. %	NADH	67 ± 7	25 ± 3
	NADH + NADPH	25 ± 3	75 ± 8

L.F.A. : Lipid bound ~~to~~ fatty acids
N.E.F.A. : Non esterified fatty acids
H.F.A. : Hydroxy fatty acids
N.H.F.A. : Non hydroxy fatty acids

Values are expressed as percentage of total extracted radioactivity.

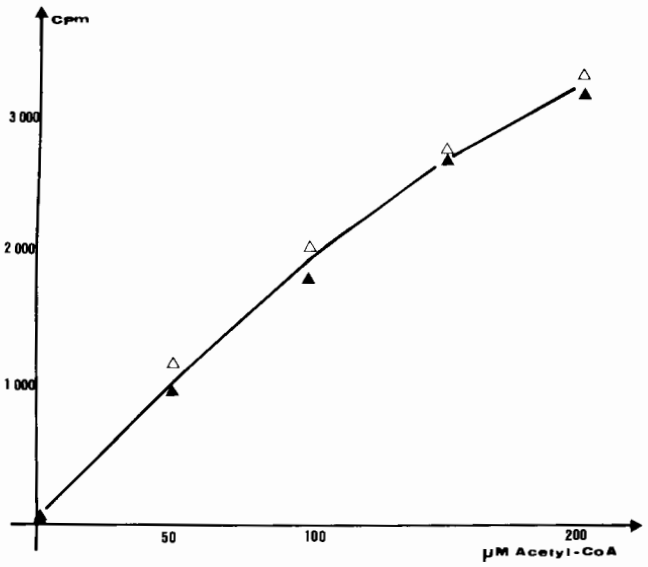

Figure 3 : Incorporation of acetyl-CoA into palmityl-CoA and stearyl-CoA in the presence of varying concentration of radioactive acetyl-CoA.
Palmityl-CoA : △ Stearyl-CoA : ▲

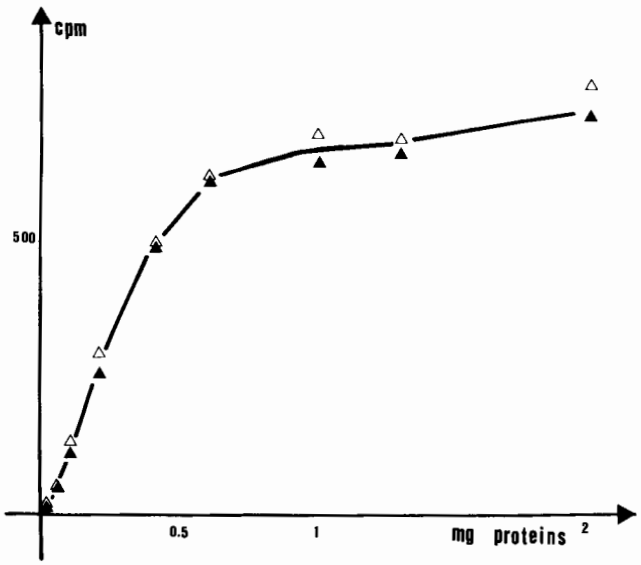

Figure 4 : Effect of increasing concentration of incubated mitochondrial proteins on palmityl-CoA and stearyl-CoA elongation.
Palmityl-CoA : △ Stearyl-CoA : ▲

NADPH (500 µM), the elongation enzymes provide mainly non hydroxy fatty acids. With NADH alone, more than 60 % of the fatty acids are hydroxylated. Fatty acids bound to lipids and non esterified fatty acids have the same profile (Table I).

When the assay consists in adding varying concentration of radioactive acetyl-CoA to a constant quantity of acyl-CoA in the incubation medium (Figure 3) or in adding varying concentration of mitochondrial proteins (Figure 4) to the complete incubation medium, similar kinetics are obtained for palmityl-CoA and stearyl-CoA.

DISCUSSION

This study further demonstrates that palmityl-CoA elongation is effective in mouse brain mitochondria (18). Acetyl-CoA is found to be the two-carbon unit donor in the presence of NADH and NADPH, thus providing hydroxy and non hydroxy (two-carbon longer) fatty acids. NADPH is necessary to obtain non hydroxylated fatty acids in brain as in liver (26). For stearyl-CoA elongation, the same cofactors and substrates are required. Palmityl-CoA provides C_{18} and stearyl-CoA provides C_{20}.

Evidence is presented that in brain mitochondria both acyl-CoA are elongated by the same enzyme system, unlike in microsomes : in brain microsomes we have demonstrated (2, 27-30) that at least three different enzyme systems are involved in fatty acid biosynthesis : a "de novo" system providing mainly C_{16} and two elongating systems, using malonyl-CoA as the two-carbon unit donor and NADPH alone ; one provides C_{18} from C_{16} and the other elongates stearyl-CoA to very long chain fatty acids with different patterns of enzymatic activity.

This difference between the two cellular organelles may involve a different regulation as the synthesized long chain fatty acids have a different fate according to their origin : we have shown that microsomal long chain fatty acids are incorporated into myelin sheath lipids and that mitochondrial elongation is not related to myelination (31).

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