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INTRACELLULAR DISTRIBUTION OF PALMITOYL-CoA SYNTHETASE IN RAT LIVER

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SUMMARY

1. The intracellular localization of rat-liver long-chain fatty acyl-CoA synthetase (acid: CoA ligase (AMP), EC 6.2.1.3; trivial name: palmitoyl-CoA synthetase) has been studied. Our results fail to confirm a previous report that the plasma membrane fraction is richest in palmitoyl-CoA synthetase[1]. In agreement with FARSTAD *et al.*[2] we found most of the activity in the microsomes, some activity in the mitochondria, and only traces in the nuclear-*plus*-cell-debris fraction, which contained the plasma membrane *plus* contaminants from other fractions.

2. The cell debris was separated from the nuclei and fractionated on a sucrose gradient. Each of the fractions was characterized by enzyme markers; DNA and protein content; and palmitoyl-CoA synthetase activity. The specific activity of palmitoyl-CoA synthetase was highest in those gradient fractions which had a high specific activity of the microsomal or mitochondrial marker enzymes. The specific activity of palmitoyl-CoA synthetase in plasma membranes isolated by two different methods was only 25% of the corresponding microsomal specific activity.

INTRODUCTION

Two different intracellular localizations of long-chain fatty acyl-CoA synthetase (acid: CoA ligase (AMP), EC 6.2.1.3; trivial name: palmitoyl-CoA synthetase) have been published recently. PANDE AND MEAD[1] found 62% of the enzymatic activity in the nuclear-*plus*-cell-debris fraction. The activity was reported to be associated with the debris fraction which was separated from the nuclei by flotation in 2.2 M sucrose. Lesser activity was found in the microsomes and mitochondria. FARSTAD *et al.*[2], however, reported that 70% of the synthetase activity was located in the microsomes and 30% in the mitochondria, with little or no activity in the other fractions. The present investigation attempted to resolve these conflicting reports.

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MATERIALS AND METHODS

Materials

AMP, D-glucose 6-phosphate, and deoxyguanosine were purchased from Sigma Chemical Company; ATP from Boehringer-Mannheim; CoA from Pabst Laboratories, palmitic acid from Applied Science Labs; L-glutamic acid from Nutritional Biochemicals Corp. Other chemicals were A.R. grades.

Methods

Tissue fractions. Five fed male Sprague–Dawley rats were killed by decapitation, and the livers were homogenized at 1300 rev./min by three down-and-up strokes of the Teflon pestle in the glass tube. The radial clearance between the pestle and the glass tube was 0.051–0.076 mm. The homogenate was fractionated essentially according to DE DUVE *et al.*[3], as follows: (Fig. 1). The whole homogenate, W, was centrifuged at $600 \times g$ for 10 min, and the pellet was washed once by rehomogenization in 0.4 vol. of 0.25 M sucrose and recentrifugation as before. The washed pellet of nuclei *plus* cell debris, N, was retained, and supernatant and wash were combined to form

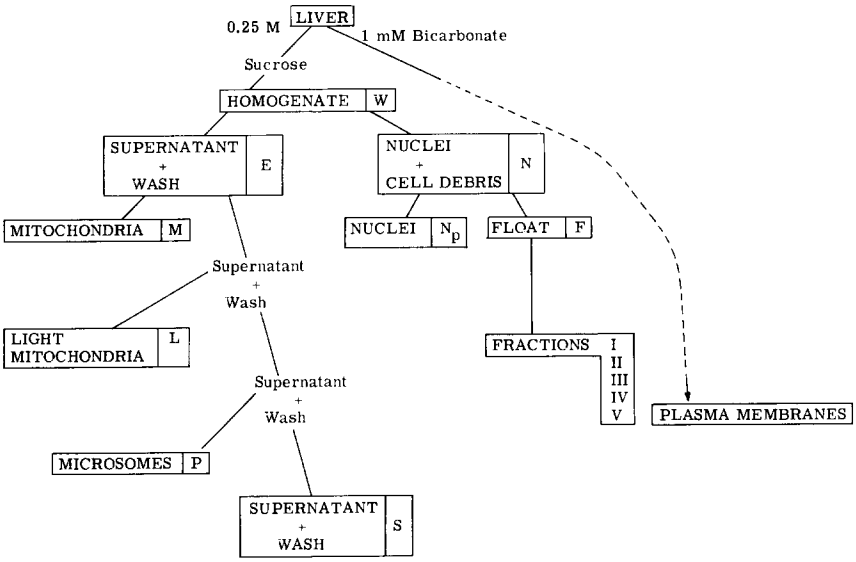

Fig. 1. Fractionation of rat liver. Flow sheet.

the cytoplasmic extract, E. The latter was fractionated into a heavy mitochondrial fraction, M; a light mitochondrial fraction, L; a microsomal fraction, P; and a final supernatant, S; by successive centrifugations at $3300 \times g$ for 10 min, $25\,000 \times g$ for 10 min and $105\,000 \times g$ for 60 min. (All g values given are $g_{av.}$). Each fraction was washed once and the washings combined with the supernatant at each stage. This procedure was based on the $g \cdot$min integrated forces of DE DUVE *et al.*[3], except that $6300\,000 \times g \cdot$min were used to sediment the microsomal fraction.

The pellet of nuclei *plus* cell debris was fractionated in 2.2 M sucrose into a

nuclear pellet, N_p, and a floating fraction, F, according to the method of CHAUVEAU *et al.*[4]. The Floating fraction F was further fractionated on a linear sucrose density gradient according to the method of NEVILLE[5], into either four or five fractions I, II, III, IV, V, from the top of the tube to the interface of the gradient and the 50% sucrose cushion. Each of these fractions was assayed for palmitoyl-CoA synthetase activity, marker enzyme activity, DNA, and protein. In separate experiments plasma membranes were prepared directly by homogenization in 1 mM sodium bicarbonate according to NEVILLE[5].

Assay of palmitoyl-CoA synthetase, marker enzymes, DNA, and protein. Palmitoyl-CoA synthetase was determined according to PANDE AND MEAD[1], using 4.0 mM palmitate and a 1-h incubation at 37°. The reaction was linear for 1 h at the protein concentrations used. Assays were run usually with 100 or 200 μg protein per 0.5 ml assay; and activities were proportional to the amount of protein used.

Glucose-6-phosphatase (a microsomal marker[3,6]) was assayed by the method of

TABLE I

INTRACELLULAR DISTRIBUTION OF PALMITOYL-CoA SYNTHETASE, MARKER ENZYMES AND CHEMICAL CONTENTS OF RAT LIVER

W = homogenate, E = cytoplasmic extract, N = nuclear, M = heavy mitochondria, L = light mitochondria, P = microsomes, S = supernatant. Absolute values for whole homogenate, W, or total homogenate, E + N, are expressed in μmoles/g wet wt. per h at 37° (glutamate dehydrogenase, 25°). Specific activities (in parentheses) are expressed in μmoles/mg protein per h at 37° (glutamate dehydrogenase, 25°).

Enzyme	*Absolute values*		*Values (%)*							*Recovery (%)*
	Whole homogenate (W)	*Total homogenate (E + N)*	*W*	*E+N*	*N*	*M*	*L*	*P*	*S*	
Protein (mg/g wet wt.)	—	165	—	100	12.8	21.4	3.7	18.2	35.6	91.7
DNA (μg/mg protein)	—	9.2	—	100	79.0	3.2	0.4	1.2	3.4	87.2
Palmitoyl-CoA synthetase	330	—	100 (1.8)	—	12.4 (2.0)	21.4 (2.0)	3.6 (2.0)	42.2 (4.6)	1.7 (0.1)	81.3
Palmitoyl-CoA synthetase (Data of PANDE AND MEAD[1])	858	—	100 (4.7)	—	62 (14.4)	13 (1.9)		25 (8.7)	3 (0.4)	103
Palmitoyl-CoA synthetase*	—	397	—	100	10.4	17.8	3.0	35.1	1.4	67.7
Palmitoyl-CoA synthetase (Data of FARSTAD *et al.*[2])	—	86.4	—	100	4.9	17.5	0.4	41.5	3.7	67.5
5′-Nucleotidase	—	612	—	100 (3.7)	45.6 (13.3)	15.4 (2.7)	10.8 (10.8)	26.6 (5.4)	10.4 (1.1)	108.8
Glutamate dehydrogenase	—	282	—	100 (1.7)	10.6 (1.4)	60 (4.8)	2.0 (0.9)	2.5 (0.2)	0.6 (0.03)	75.7
Glucose-6-phosphatase	—	960	—	100 (5.8)	10.8 (5.0)	9.6 (2.6)	6.0 (9.4)	48.3 (15.5)	2.0 (0.3)	76.7

* Same results as Line 3 expressed using total homogenate, E + N, as 100% activity instead of whole homogenate, W.

STAHL AND TRAMS[7]; and 5'-nucleotidase (a plasma-membrane marker[8]) according to EMMELOT AND BOS[9], at pH 7.4 in a 1.0-ml volume. For both enzymes 100-μg samples of protein were used; and after incubation at 37° for 5 and 10 min the reaction was stopped with 0.5 vol. of 15% trichloroacetic acid, and P_i in the supernatant was determined by the method of AMES AND DUBIN[10]. Glutamate dehydrogenase (a mitochondrial marker[11,6]) was assayed for the forward reaction *i.e.* NAD^+ reduction, by the method of BEAUFAY *et al.*[11].

DNA was determined by the diphenylamine procedure[12] and protein by the method of LOWRY *et al.*[13].

RESULTS AND DISCUSSION

The distribution of marker enzymes, DNA and protein, and palmitoyl-CoA synthetase in subcellular fractions from rat liver is summarized in Table I. The intracellular distributions of palmitoyl-CoA synthetase previously reported[1,2] are included for comparison. The specific activities of palmitoyl-CoA synthetase, marker enzymes, and the DNA/protein ratio in fractions of the cell debris prepared by sucrose density gradient centrifugation, and in plasma membranes prepared by the method of NEVILLE[5] are summarized in Table II. The data shown in Table I and in Expt. 2 of Table II are from the same fractionation experiment.

We observed an intracellular distribution of palmitoyl-CoA synthetase very similar to the one reported by FARSTAD *et al.*[2]. Most of the enzyme activity was in the microsomal and mitochondrial fractions; and only 10% was found in the nuclei *plus* cell debris before correcting for cross contamination by other cell fractions. Our results do not confirm the suggestion of PANDE AND MEAD[1] that palmitoyl-CoA

TABLE II

SPECIFIC ACTIVITIES OF PALMITOYL-CoA SYNTHETASE, AND MARKER ENZYMES, IN FRACTIONS OF CELL DEBRIS, AND IN PLASMA MEMBRANE PREPARATIONS

Specific activities are expressed in μmoles/mg protein per h at 37° (25° for glutamate dehydrogenase). DNA is expressed in terms of μg DNA/mg protein. Plasma membranes were isolated either directly by the procedure of NEVILLE[5] (Expts. 3, 4); or by NEVILLE[5] fractionation of cell debris in the nuclear float[4] (Expts. 1, 2).

Expt. No.	*Fraction*		*Palmitoyl-CoA synthetase*	*5'Nucleotidase*	*Glutamate dehydrogenase*	*Glucose-6-phosphatase*	*DNA*
1	Gradient fractions of cell debris	I	5.5	8.5	0.6	8.3	2.0
		II	5.0	1.5	3.0	1.2	1.3
		III	2.6	18.8	1.1	0.9	27.8
	(Plasma membrane)	IV	1.3	26.3	0.8	1.3	56.6
2	Gradient fractions of cell debris	I	2.8	0.9	0.4	1.2	6.7
		II	3.4	9.9	1.6	3.4	5.1
		III	3.5	6.2	3.6	5.5	5.1
		IV	2.5	11.9	2.7	1.6	16.3
	(Plasma membrane)	V	0.9	23.2	0.6	1.3	63.9
	Nuclear pellet N_p		0.9	4.8	0.4	1.0	394.0
3	Plasma membranes		1.3	–	–	–	–
	Plasma membranes		1.1	–	–	1.4	–

synthetase activity in rat liver is localized predominantly in the cell membranes. PANDE AND MEAD[1] separated a nuclear pellet from a float of cell debris and found that the float had high palmitoyl-CoA synthetase activity. This centrifugation procedure was primarily designed to isolate pure nuclei, and the float of cell debris in which the palmitoyl-CoA synthetase activity was found is probably heterogeneous[4]. However marker enzyme studies were not offered to support the contention that palmitoyl-CoA synthetase was associated with plasma membranes. In addition to plasma membranes we found that the cell debris isolated by flotation in 2.2 M sucrose contained substantial amounts of microsomal and mitochondrial fragments as determined by marker enzyme studies. We also found that after fractionation of the cell debris on a linear sucrose gradient, the specific activity of palmitoyl-CoA synthetase in Fractions II and III was approx. 4 times greater than the corresponding value for the plasma membrane Fraction V. (Table II, Expt. 2). These Fractions II and III, also had high specific activities of the microsomal and mitochondrial marker enzymes. Our data show that palmitoyl-CoA synthetase activity in the cell debris appears to be associated predominantly with microsomal and mitochondrial contaminants and not with the plasma membranes. The specific activity of palmitoyl-CoA synthetase in plasma membranes isolated according to NEVILLE[5] also had only 25% of the microsomal specific activity (compare Tables I and II). Whether the low activity of palmitoyl-CoA synthetase in the plasma membrane preparations is an authentic plasma membrane activity, or is due to microsomal and mitochondrial contamination is uncertain. EMMELOT *et al.*[14] maintain that the presence of microsomal enzymes with low specific activity in plasma membrane preparations does not necessarily indicate contamination from microsomes. They postulate that a functional continuity may exist between the plasma membrane and membranes of the endoplasmic reticulum.

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REFERENCES

1 S. V. PANDE AND J. F. MEAD, *J. Biol. Chem.*, 243 (1968) 352.
2 M. FARSTAD, J. BREMER AND K. R. NORUM, *Biochim. Biophys, Acta*, 132 (1967) 492.
3 C. DE DUVE, B. C. PRESSMAN, R. GIANETTO, R. WATTIAUX AND F. APPELMANS, *Biochem. J.*, 60 (1955) 604.
4 J. CHAUVEAU, Y. MOULE AND CH. ROUILLER, *Exptl. Cell Res.*, 11 (1956) 317.
5 D. M. NEVILLE, JR., *Biochim. Biophys. Acta*, 154 (1968) 540.
6 C. DE DUVE, *J. Theoret. Biol.*, 6 (1964) 33.
7 W. L. STAHL AND E. G. TRAMS, *Biochim. Biophys. Acta*, 163 (1968) 459.
8 R. COLEMAN, R. H. MICHELL, J. B. FINEAN AND J. N. HAWTHORNE, *Biochim. Biophys. Acta*, 135 (1967) 573.
9 P. EMMELOT AND C. J. BOS, *Biochim. Biophys. Acta*, 120 (1966) 369.
10 B. N. AMES AND D. T. DUBIN, *J. Biol. Chem.*, 235 (1960) 769.
11 H. BEAUFAY, D. S. BENDALL, P. BAUDHUIN AND C. DE DUVE, *Biochem. J.*, 73 (1959) 623.
12 K. BURTON, *Biochem. J.*, 62 (1956) 315.
13 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
14 P. EMMELOT, C. J. BOS, E. L. BENEDETTI AND PH. RUMKE, *Biochim. Biophys. Acta*, 90 (1964) 126.