FORMATION OF ACIDS SHORTER THAN PALMITIC BY RAT LIVER CYTOSOL[1]

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SUMMARY: Particle-free liver supernatant of rats maintained on stock diet synthesizes fatty acids of average chain lengths 9-11 carbon atoms when the protein concentration is high while palmitic acid is the main product when protein concentration is low. This ability to synthesize acids shorter than palmitic is lost on purification of fatty acid synthetase or by starvation of the rats followed by the ingestion of a high sucrose diet. The results are consistent with the presence of a factor in the cytosol, similar to that in lactating mammary glands, which shortens the chain length of the products of fatty acid synthetase.

Rat liver fatty acid synthetase has been shown to synthesize predominantly palmitic acid (1), while the enzyme from lactating mammary gland of herbivores as well as rat synthesizes appreciable amounts of acids shorter than palmitic besides the latter (2,3,4). While attempting to establish the optimum conditions for the synthesis of palmitate by normal rat liver cytosol, required in connection with another study, it was observed that high concentrations of protein resulted in a significant decrease in the average chain length of the products. These interesting results are presented in this paper. Only the average chain lengths of the acids formed were determined in this

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study as the analysis of acids containing short chain acids that are volatile by gas-liquid chromatography is tedious and often unreliable.

MATERIALS AND METHODS

Male adult Fisher rats, weighing 250-300 g were used which were either maintained on Purina chow or were starved for two days and then refed for two days a diet containing 66% sucrose, 20% casein, 10% cellulose and 4% minerals and vitamins. Particle-free supernatant was prepared from the livers of a group of three rats. Fatty acid synthetase was purified by the method of Burton et al (1). Such a preparation from normally fed rats is not homogeneous and hence the specific activity is low (5). Protein assay, the spectrophotometric assay of enzyme and the preparation of substrates were as described earlier (6,7). For the experiments described in Table II and III the acyl-coenzyme A substrates were further purified by DEAE-cellulose chromatography (8) followed by gel filtration, thus assuring the removal of all free contaminating radioactive starting material or side products.

For the determination of chain lengths of the acids formed, parallel incubations were carried out containing labeled acetyl-CoA or labeled malonyl-CoA with the required unlabeled substrates. The acids were extracted and fractionated into butyric and a fraction consisting of hexanoic and longer chain acids (6). The average chain length of the latter fraction was computed according to the formula:

$$2 \times \frac{\text{moles of malonyl-CoA incorporated}}{\text{moles of acetyl-CoA incorporated}} + 2.$$

RESULTS AND DISCUSSION

The effect of the concentrations of malonyl-CoA and of protein on the chain lengths of the acids other than butyric are shown in Table I. The effect of malonyl-CoA concentration on the chain length of acids formed seen here is well documented (9,10). The formation of appreciable amounts of short chain acids, inferred from the average chain length, at the high protein concentration at each level of malonyl-CoA was surprising.

The results of time course studies at the two protein levels, using a 2:1 ratio of the concentrations of malonyl-CoA to acetyl-CoA, are shown in Table II which includes the respective formation of butyric acid as well. Formation of butyric acid, though significant, does not follow any particular pattern (10). The formation of the longer chain acids, on the other hand, does

TABLE I. Effect of concentration of normal cytosol protein and of malonyl-CoA on the average chain lengths of the fraction consisting of hexanoic and longer fatty acids.

Protein (mg)	Malonyl-CoA (M)	Average Chain Length (C-atoms)
1.0	1.3×10^{-4}	11.5
	6.3×10^{-4}	12.5
	1.3×10^{-3}	15.6
	6.3×10^{-3}	15.9
5.0	1.3×10^{-4}	7.9
	6.3×10^{-4}	8.9
	1.3×10^{-3}	8.6
	6.3×10^{-3}	9.7

Parallel incubations were carried out using either labeled acetyl-CoA and unlabeled malonyl-CoA or unlabeled acetyl-CoA and labeled malonyl-CoA. 0.8 ml of the incubation mixture contained potassium phosphate buffer, 6.3×10^{-3} M, pH 7.0; dithiothreitol, 5×10^{-3} M; acetyl-CoA or [1-^{14}C]-acetyl-CoA, 6.3×10^{-4} M (2.3×10^{6} CPM or 5.0×10^{6} CPM); malonyl-CoA or [2-^{14}C]-malonyl-CoA, as indicated (2.7×10^{6} or 7.2×10^{6} CPM per μmole); NADPH 6.3×10^{-3} M and protein as indicated. The incubation period was 30 min at 37°.

follow the expected pattern. At 1 mg protein level palmitic must be the major product, stearate being formed only in minor quantities (1,10). At 5 mg protein level formation of acids shorter than ten carbons in significant amounts must have occurred. The near linearity of the incorporation of both of the substrates with the incubation time, reflected in the constancy of the chain length, indicates that the formation of the short chain acids was not due to depletion of malonyl-CoA towards the patter part of the longer incubation period (Table I).

TABLE II. Effect of concentration of normal rat liver cytosol on the synthesis of butyric (C_4) and hexanoic and longer chain acid fraction at varying incubation periods.

Labeled substrate incorporated	1 mg protein (7 E.U.)* nanomoles					
	5 min		10 min		20 min	
	C_4	C_{6+}	C_4	C_{6+}	C_4	C_{6+}
Acetyl-CoA	0.1	0.6	0.2	1.2	0.2	2.2
Malonyl-CoA	1.0	3.9	2.5	8.1	4.1	15.8
	Average chain lengths (C-atoms)					
	16.1		16.1		16.3	
	5 mg protein (35 E.U.)* nanomoles					
Acetyl-CoA	1.0	2.9	1.7	5.8	3.5	11.0
Malonyl-CoA	2.5	13.0	1.8	24.6	6.0	46.5
	Average chain length					
	10.9		10.5		10.4	

Incubation conditions were as described under Table I except that the specific radioactivity of acetyl-CoA was 10×10^6 CPM/μmole and the concentration of malonyl-CoA was 1.3×10^{-3}M. The ^{14}C activity of the latter, where applicable, was 7.2×10^6CPM/μmole.

*E.U. - enzyme units; quantity of enzyme that catalyzes the oxidation of 1 nanomole of NADPH/min at 26°.

Table III presents data obtained with the synthetase at various stages of purification. The capacity to synthesize short chain acids is lost on the ammonium sulfate precipitation of the enzyme from the cytosol. Even at an enzyme concentration representing 10 mg cytosol protein the average chain length was 15.2 carbons. Similarly, starving rats and refeeding sucrose causes the loss of the ability of liver cytosol to synthesize

TABLE III. Effect of purification and sucrose-refeeding on the average chain lengths of the acids synthesized by fatty acid synthetase at high protein concentration

Enzyme source and protein	INCORPORATION [1-^{14}C]-Acetyl-Co-A	INCORPORATION [2-^{14}C]-malonyl Co-A	Average chain length (C-atoms)
	nanomoles		
1a. normal cytosol 5 mg (35 E.U.)	2.9	13.0	10.9
1b. I$(NH_4)_2SO_4$ ppt. 2.5 mg (33 E.U.)	2.7	17.4	14.9
5.0 mg (66 E.U.)	5.1	33.6	15.2
1c. Ca-phosphate gel extract, 1.5 mg (33 E.U.)	2.2	13.8	14.6
1d. II$(NH_4)_2SO_4$ ppt. 0.75 mg (31 E.U.)	1.9	12.6	15.3
1e. sucrose refed liver cytosol 0.25 mg (7.5 E.U.)	0.9	6.3	15.5
1.20 mg (36 E.U.)	6.9	47.2	15.7

Parallel incubations were done as described under Table I. Incubation period was 5 min in 1a to 1c and 10 min in Experiment 2.

acids shorter than palmitate in significant amounts.

Our results are consistent with the presence of a factor in liver, similar to that found in rabbit mammary gland (11), which binds to the synthetase with a low binding constant, and which then causes the termination of the growth of the acetyl chain long before palmitate is formed. The results further suggest that starvation and sucrose refeeding also results in the loss of this factor, perhaps by degradation.

It is possible that previous workers missed detecting the

formation of shorter acids than palmitate as care was not taken to extract and retain them during analysis and, perhaps, because of the practice of using rats starved and refed sucrose in order to obtain a high yield of fatty acid synthetase (1). Work is in progress on the identity of the acids formed and the physiological role, if any, of the chain terminating factor.

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