INFLUENCE OF LECITHIN ON THE ACTIVITY OF THE GTP-DEPENDENT ACYL-CoA SYNTHETASE

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A GTP-dependent acyl-CoA synthetase, which is active with short-chain fatty acid substrates only, was isolated from buffered extracts of an acetone powder of beef liver mitochondria (Rossi and Gibson, 1964):

$$GTP + RCOOH + CoASH \rightleftharpoons RCOSCoA + GDP + P_i$$

Recently, a similar synthetase, but active with both long- and short-chain fatty acids, has been purified by a different procedure (not involving acetone from rat liver mitochondria (Galzigna, Rossi, Sartorelli, and Gibson, 1966). It seemed possible that organic solvent treatment might explain the difference in substrate specificity between these two preparations. The studies reported in the present communication indicate that protein-bound lipid is necessary for full activity of the GTP-dependent synthetase system.

The enzyme purification procedure (Galzigna, Sartorelli, Rossi, and Gibson, in press) involved sonication of rat liver mitochondria in Triton X-100; high-speed centrifugation to remove insoluble protein; acid pH treatment; calcium-phosphate gel adsorption; and Dowex-50 chromatography. Mitochondria were separated from rat liver according to Schneider and Hogeboom (1950). En-

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zyme activity was measured with the nitroprusside method (Grunert and Phillips, 1951) and by following inorganic phosphate release (Berenblum and Chain, 1938). In the nitroprusside assay for SH disappearance, the following reagents were incubated in 0.20 ml final volume for 15 minutes at 38°C: Tris-HCl (pH 7.4), 100 µmoles; $MgCl_2$, 2.5 µmoles; KBH_4, 1 µmole; potassium oleate (butyrate), 1 µmole; CoASH, 0.40 µmole; GTP, 1 µmole; and 30-100 µg of enzyme. For measuring inorganic phosphate release the following were incubated in 0.50 ml for 15 minutes at 38°C: Tris-HCl (pH 7.4), 100 µmoles; $MgCl_2$, 7.5 µmoles; glutathione, 16 µmoles; potassium oleate (butyrate), 3 µmoles; albumin (bovine serum, crystalline), 6 mg; CoASH, 1 µmole; GTP, 1 µmole; and 0.5-1.0 mg enzyme.

Serial extraction of the lyophilized, purified enzyme with 90% acetone in water resulted in a progressive loss of specific enzyme activity with potassium butyrate or oleate as substrate (Fig. 1). The lyophilized preparation was employed to minimize non-specific protein denaturation (Szarkowska, 1966). In this study 16 mg of enzyme (biuret) were extracted three times with 10 ml of

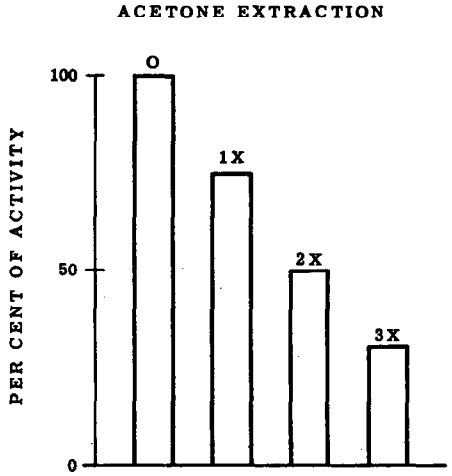

Fig. 1. Percent of initial specific enzyme activity following acetone extraction of lyophilized enzyme. The full specific activity (100%) with potassium oleate as substrate was - Δ 1.5 µmoles SH/mg protein/hour (or + Δ µmoles of P_i/mg protein/hour). With potassium butyrate the initial specific activity (before extraction) was - Δ 1.8 µmoles SH/mg protein/hour (or + Δ µmoles P_i/mg protein/hour).

90% acetone at room temperature. Twenty-five percent of the initial specific activity remained.

The lyophilized powder which was extracted three times could be restored to 80% of the initial full activity by preincubating the redissolved protein for thirty minutes at 0°C with pure egg lecithin (L) or with the acetone lipid extract (A. E.) of the enzyme (after removal of acetone) (Fig. 2).

If fresh liver mitochondria (Schneider and Hogeboom, 1950) are pretreated with 90% acetone in water, according to Fleischer et al (1962), the specific activity of the sonicated extract is diminished 50% when long-chain fatty acids are used as substrates. Short-chain fatty acid activation (butyrate) is unaffected under these conditions (Table I). Preincubation of the sonicated extract with 0.46 mg of egg lecithin micelles per mg of protein at 0°C for 30 minutes partially restores the capacity for oleate activation (Table I).

EFFECT OF PHOSPHOLIPIDS ON THE ACTIVITY OF ACETONE EXTRACTED ENZYME

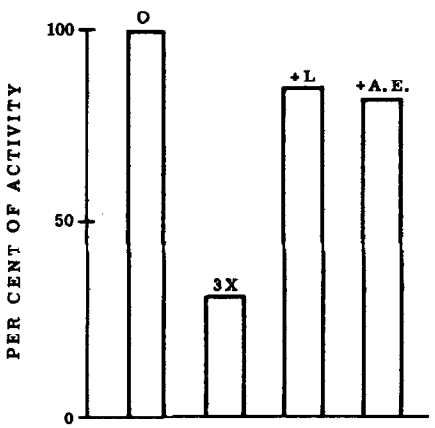

Fig. 2. Percent of initial specific activity (SH disappearance) restored after pretreatment of acetone-extracted enzyme with pure egg lecithin (+ L), or with the acetone extract (+ A. E.). 0.5 mg of micellar lecithin, dispersed by sonication in 0.1 ml of 0.1 M Tris-HCl (pH 7.4), was preincubated with 0.86 mg of lipid-extracted enzyme in a total volume of 0.2 ml for 30 minutes at 0°C. Similarly, the lipid extract (after removal of acetone) from 16 mg (biuret) of lyophilized enzyme was added back to 0.86 mg (biuret) of the extracted enzyme under the same conditions. The acetone extract of the purified enzyme, as well as chloroform-methanol extracts, contained only lecithin by thin layer chromatography (method of Wagner, 1961).

TABLE I

Effect of the removal and addition of lecithin on the activation of fatty acids in sonicated extracts of mitochondria. Specific activity is expressed as - Δ μmoles SH/mg protein/hour. 500 μg of protein (sonicated extract of mitochondria) were employed in a total incubation volume of 0.20 ml.

	Oleate	%	Butyrate	%
1. Sonicated extract of mitochondria	.800	100	.700	100
2. Sonicated extract of mitochondria pretreated with acetone-water	.400	50	.680	94
3. Same + lecithin	.600	75	.700	100

In recent years the importance of structural lipids in determining the activity of certain enzymes has been brought to light (Green and Fleischer, 1963; Brierley, Merola and Fleischer, 1962; Jurtshuk, Sekuzu and Green, 1961). Similarly the results reported in the present paper show that GTP-dependent acyl-CoA synthetase activity is affected by removal and addition of lecithin. Chain length specificity may also depend on the presence of bound lecithin. Lecithin, bound to protein by secondary bonds (Goodman and Shafrir, 1959) may be involved directly in orienting the fatty acid substrate, or indirectly, by influencing the conformation of the enzyme.

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