Short Communications

Chain-Length Specificity for Termination of Fatty Acid Biosynthesis by Fatty Acid Synthetase Complexes from Lactating Rabbit Mammary Gland and Rat Liver

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Fatty acid synthetase, which catalyses the synthesis of saturated even-numbered fatty acids from acetyl-CoA, malonyl-CoA and NADPH, has been isolated as a multienzyme complex from rat liver (Burton, Haavik & Porter, 1968) and adipose tissue (Martin, Horning & Vagelos, 1961). Both these mammalian enzyme complexes synthesized long-chain fatty acids (predominantly $C_{16:0}$). This chain-length specificity for termination of fatty acid synthesis may be reflected in the fact that rat liver (Getz, Bartley, Stirpe, Notton & Renshaw, 1961) and adipose tissue (Smith, Watts & Dils, 1968) do not contain significant proportions of esterified short-chain ($C_{4:0}$ – $C_{8:0}$) or medium-chain ($C_{10:0}$ – $C_{14:0}$) fatty acids.

In contrast, milk from a number of species contains a high proportion of short-chain and medium-chain fatty acids (Garton, 1963; Glass, Troolin & Jenness, 1967). In rabbit milk 70% (molar) of the esterified fatty acids are $C_{8:0}$ and $C_{10:0}$ and these are not dietary in origin (Smith, *et al.* 1968). We have therefore purified the fatty acid synthetase complex from lactating rabbit mammary gland and investigated whether the chain-length specificity for termination of fatty acid synthesis is different from that of the rat liver enzyme complex. The latter was chosen for this comparison since it is the only mammalian fatty acid synthetase complex to have been extensively characterized (Burton *et al.* 1968).

Methods. Particle-free supernatant fractions from lactating rabbit mammary gland and rat liver were prepared as described previously (Smith, Easter & Dils, 1966; Easter & Dils, 1968) except that 0.3M-sucrose containing 1mM-dithiothreitol and 1mM-EDTA was used as homogenization medium. Purification of the rabbit mammary-gland fatty acid synthetase complex from the particle-free supernatant fraction was based on the method of Hsu, Wasson & Porter (1965) for the preparation of the pigeon liver enzyme complex. The method was modified by maintaining protein solutions at 1mM with respect to both dithiothreitol and EDTA, and

at pH 7.0, using a calcium phosphate gel/protein dry-weight ratio 1:1 and a linear gradient of potassium phosphate buffer (0.05–0.25M) for ion-exchange chromatography. Fatty acid synthetase complex from the livers of fed rats was purified from the particle-free supernatant fraction by the method of Burton *et al.* (1968), but modified as described above, except that the gel/protein ratio was 1:2.

The fatty acid synthetase complex was assayed spectrophotometrically (Wakil, Titchener & Gibson, 1958; Lynen, Hopper-Kessel & Eggerer, 1964). Optimum assay conditions (final vol. 1ml) were 200mM-potassium phosphate buffer, pH 6.6, 1mM-dithiothreitol, 1mM-EDTA, 30 μ M-acetyl-CoA, 40–50 μ M-malonyl-CoA, 240 μ M-NADPH and enzyme protein. After measurement of oxidation in the absence of acetyl-CoA and malonyl-CoA, the reaction was started by the addition of CoA esters. Reaction rate was linear with time for at least 3min at 37°C.

The pattern of fatty acids synthesized by the purified enzyme complex was determined by measuring the incorporation of [$1-^{14}C$]acetyl-CoA. The incubation conditions used are described in Table 1. After the reaction had been stopped, carrier fatty acids (200nmol each of $C_{4:0}$ – $C_{18:0}$ acids) were added. After alkaline hydrolysis, carrier and synthesized fatty acids were extracted and analysed by gas-liquid radiochromatography as free acids (Smith & Dils, 1965). Loss of volatile fatty acids was determined by comparing the mass chromatograph of the extract with that of the carrier mixture. The radioactivity in each fatty acid (obtained from the radioactivity chromatogram) was corrected for this loss.

The $s_{20,w}^0$ of the rabbit mammary-gland fatty acid synthetase complex was determined by the method of Schachman (1957). Sedimentation studies were carried out at 4°C and 42040 rev./min (Spinco model E ultracentrifuge) in 0.25M-potassium phosphate buffer, pH 7.0, 1mM-dithiothreitol and 1mM-EDTA with protein concentrations from 3

Table 1. *Effect of malonyl-CoA on the pattern of fatty acids synthesized by fatty acid synthetase complex*

Incubations (15 min at 37°C, final vol. 1.0 ml) contained (a) 200 mM-potassium phosphate buffer, pH 6.6, 1 mM-dithiothreitol, 1 mM-EDTA, 30 μ M-[1- 14 C]acetyl-CoA (20 μ Ci/ μ mol), 480 μ M-NADPH, malonyl-CoA as shown and 8 μ g of purified rabbit mammary-gland fatty acid synthetase complex (specific activity 880 nmol of NADPH oxidized/min per mg of protein, as determined by spectrophotometric assay); (b) as in (a) except that 10 μ M-[1- 14 C]acetyl-CoA (20 μ Ci/ μ mol) and 2 μ g of purified rat liver fatty acid synthetase complex (specific activity 580 nmol of NADPH oxidized/min per mg of protein) were used. NADPH oxidation showed that reaction rate was linear for 15 min under optimum conditions. The symbol + means that the fatty acid was detected, but contained less than 1% of the incorporated radioactivity.

Source of fatty acid synthetase complex	Concn. of malonyl-CoA (μ M)	% of radioactivity incorporated into								Total [1- 14 C]acetyl-CoA incorporated (nmol)
		C _{4:0} acid	C _{6:0} acid	C _{8:0} acid	C _{10:0} acid	C _{12:0} acid	C _{14:0} acid	C _{16:0} acid	C _{18:0} acid	
(a) Rabbit mammary gland	3	25	5	5	3	5	20	37	+	0.6
Rabbit mammary gland	12	48	2	+	1	1	10	38	+	2.3
Rabbit mammary gland	35	16	2	+	1	1	7	71	2	4.2
Rabbit mammary gland	120	14	1	1	+	1	2	75	6	2.4
(b) Rat liver	1	28	9	2	2	7	24	26	2	0.2
Rat liver	10	20	2	1	1	1	6	66	3	1.1
Rat liver	100	8	2	+	+	2	4	88	1	0.4

to 11 mg/ml. Protein was determined as described by Smith & Dils (1966) or by the method of Lowry, Rosebrough, Farr & Randall (1951).

Results and discussion. Purified rabbit mammary-gland fatty acid synthetase complex had specific activities of 880 ± 30 nmol of NADPH oxidized/min per mg of protein (mean $\pm$ S.E.M. for 15 preparations). The homogeneity of the freshly prepared enzyme was established by using the criteria of sedimentation as a single symmetrical peak on analytical ultracentrifugation and elution as a single protein peak with constant specific activity from Whatman DE-52 DEAE-cellulose and Sephadex G-200. An $s_{20,w}^0$ value of 16.5 ± 0.2 S (mean $\pm$ S.E.M. for three preparations) was determined from sedimentation-velocity studies.

The effect of increasing malonyl-CoA concentrations on the pattern of fatty acids synthesized by the rabbit mammary-gland and rat liver fatty acid synthetase complexes is shown in Table 1. Both complexes synthesized an identical range (C_{4:0}–C_{18:0}) of fatty acids. The major product at maximum rates of synthesis was C_{16:0} acid, which is in agreement with previous results for mammalian fatty acid synthetase complexes (Martin *et al.* 1961; Burton *et al.* 1968).

With both complexes C_{4:0} acid was synthesized at all malonyl-CoA concentrations used. Failure of other workers (Burton *et al.* 1968) to detect short-chain fatty acids synthesized by the purified rat liver enzyme complex was probably due to methylation of the volatile fatty acids synthesized and loss of these methyl esters on evaporation to dryness (Smith & Dils, 1965) before analysis. Though C_{4:0} acid is present (10–20% molar) in ruminant milk triglycerides (Garton, 1963; Glass *et al.* 1967),

it has not so far been identified as a component of rabbit milk (Smith *et al.* 1968) or rat liver (Getz *et al.* 1961) triglycerides. Accumulation *in vivo* in an esterified form of C_{4:0} acid synthesized by the fatty acid synthetase complex may be prevented by its rapid oxidation (Jowett & Quastel, 1935) or chain elongation.

The similarity in the patterns of fatty acids synthesized by the complexes was very marked, considering the difference in fatty acid composition of rabbit milk and rat liver triglyceride. Isolated rabbit mammary-gland fatty acid synthetase complex does not synthesize the high proportion of C_{8:0} and C_{10:0} acids found in rabbit milk, since synthesized medium-chain fatty acids appear to be rapidly elongated by the enzyme complex and so do not accumulate.

The variation in the pattern of fatty acids synthesized by each complex was dependent on the concentration of malonyl-CoA rather than on the rate of synthesis. The acyl moiety of both acetyl-CoA and malonyl-CoA is capable of binding to the thiol group of 4'-phosphopantetheine of fatty acid synthetase complexes from yeast (Lynen, Oesterhelt, Schweizer & Willecke, 1968) and pigeon liver (Jacob, Butterworth & Porter, 1968). For both rabbit mammary-gland and rat liver enzyme complexes competition between acetyl-CoA and malonyl-CoA for this acyl-binding site (the site of the chain-elongation reaction) could account for the observed shift in the chain length of synthesized fatty acids. At low malonyl-CoA concentrations the binding of the 'primer' acetyl moiety may be favoured, thereby increasing the probability of termination of chain elongation by displacement of the growing acyl chain. This has also been suggested

by Lynen *et al.* (1964) for yeast fatty acid synthetase complex, where the proportions of fatty acids synthesized are also modified by substrate concentrations. Competition between substrates also agrees with the kinetic model of Sumper, Oesterhelt, Riepertinger & Lynen (1969), where the malonyl-CoA/acetyl-CoA concentration ratio is considered as a factor influencing the chain length of products.

From the similarity of fatty acid patterns and their variation with malonyl-CoA concentration we conclude that the specificity of termination of the chain-lengthening process must be similar for both rabbit mammary-gland and rat liver fatty acid synthetase complexes. It is possible that all mammalian fatty acid synthetase complexes have similar specificities for chain termination, and have no inherent mechanism for the synthesis of a characteristic pattern of products.

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