PARTIAL PURIFICATION FROM RABBIT MAMMARY GLAND OF A FACTOR WHICH CONTROLS THE CHAIN LENGTH OF FATTY ACIDS SYNTHESISED

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SUMMARY: A factor which controls the chain length of synthesised fatty acids has been partially purified from the cytosol of lactating rabbit mammary gland. The factor contains protein and has a molecular weight of 40,000-50,000. It alters the fatty acids synthesised by purified fatty acid synthetase from mainly $C_{14:0}$ and $C_{16:0}$ acids to $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$ acids. These medium-chain fatty acids are characteristic of rabbit milk.

Lactating rabbit mammary gland synthesises predominantly octanoic ($C_{8:0}$) and decanoic ($C_{10:0}$) acids both *in vivo* (1) and *in vitro* (2). By contrast, the purified fatty acid synthetase complex from this tissue synthesises short-chain ($C_{4:0}$ and $C_{6:0}$) and long-chain ($C_{14:0}$ and $C_{16:0}$) fatty acids. The complex only synthesised medium-chain acids in amounts which are approximately equimolar to the fatty acid synthetase. This amount cannot be increased by factors, such as substrate concentrations, which control the relative proportions of short- and long-chain acids synthesised (3).

The microsomal fraction from rabbit mammary gland cannot of itself induce the synthesis of medium-chain fatty acids by purified fatty acid synthetase (4). However, there is evidence that the particle-free supernatant fraction of this tissue contains an unidentified factor which terminates fatty acid synthesis at $C_{8:0}$-$C_{12:0}$ acids (4-7). This paper describes the partial purification from the particle-free supernatant of a protein-containing factor which alters the specificity of fatty acid synthetase to favour the synthesis of these medium-chain acids.

MATERIALS AND METHODS

Subcellular fractions of mammary gland from lactating rabbits (12-16 days

post partum) were prepared (4). Portions of the particle-free supernatant fraction (about 50 ml; 20 mg protein/ml) were applied to a column (5 cm x 74 cm) of Sephadex G-100 and eluted with 0.1 M potassium phosphate buffer, pH 7.0, which contained 1 mM dithiothreitol at a rate of 90 ml/min. The eluate was monitored at 280 nm. Appropriate fractions (13.5 ml) were pooled and concentrated to about 12 ml using an Amicon Ultrafiltrator (Type CEC1) with a PM-10 filter.

The incubation conditions used to test the effectiveness of the Sephadex fractions in inducing the synthesis of medium-chain fatty acids are described in the legends to the Tables. The synthesised lipids were separated by thin-layer chromatography and the fatty acids in the triglyceride fraction were analysed by gas-liquid radiochromatography. In addition, the total lipids synthesised were saponified and the total fatty acids analysed by gas-liquid radiochromatography (4).

Fatty acid synthetase was purified from lactating rabbit mammary gland (3). Protein was determined by the method of Lowry (9).

RESULTS AND DISCUSSION

The elution pattern on Sephadex G-100 of the particle-free supernatant is shown in Fig. 1.

Fatty acid synthesis was measured from [1-^{14}C]acetate in the presence of

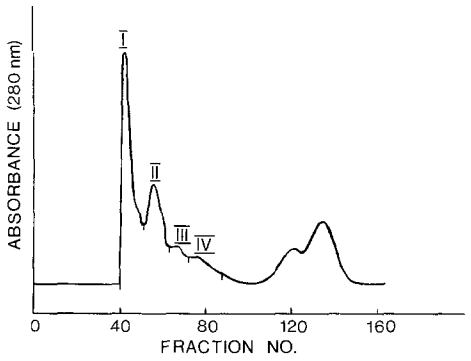

Fig. 1. Sephadex G-100 chromatography of the particle-free supernatant fraction from lactating rabbit mammary gland. Fractions I to IV were collected as shown.

Table 1. The effect of Sephadex G-100 fractions on the pattern of fatty acids synthesised from [1-^{14}C]acetate by Fraction I plus the microsomal fraction

Addition	Lipid analysed*	Mole % fatty acids synthesised							Total nmole acetate incorporated
		4:0	6:0	8:0	10:0	12:0	14:0	16:0	
None	TG	12	5	4	6	10	42	21	7
	TFA	48	10	3	4	6	20	9	27
Fraction II	TG	17	4	6	7	8	29	30	142
	TFA	27	11	6	5	5	23	23	310
Fraction III	TG	19	7	9	26	18	17	4	42
	TFA	39	12	7	16	10	13	3	106
Fraction IV	TG	0	0	28	58	12	2	0	6
	TFA	47	10	20	19	3	1	0	30
Albumin	TG	22	17	3	3	2	25	28	11
	TFA	42	14	1	1	1	17	24	47

*TG = triglycerides; TFA = total fatty acids.
The incubation system (1.0 ml) contained 50 mM potassium phosphate buffer, pH 7.2, 1 mM dithiothreitol, 10 mM $MgCl_2$, 5 mM potassium citrate, 10 mM $NaHCO_3$, 10 mM ATP, 0.15 mM CoA, 0.5 mM NAD^+, 0.05 mM $NADP^+$, 10 mM glucose 6-phosphate, 5 mM rac-glycerol 3-phosphate and 1.5 mM potassium [1-^{14}C]acetate (1.60 μCi/μmole). Fraction I (1.16 mg protein) from the Sephadex column (see Fig. 1) and 1.31 mg of microsomal protein from lactating rabbit mammary gland were used as the source of enzymes. Fraction II (2.90 mg protein), III (0.37 mg protein) or IV (0.65 mg protein) from the Sephadex column or bovine serum albumin (4.0 mg) was added as shown. Incubations were for 30 minutes at 37°C.

Fraction I plus the microsomal fraction from the gland. This resulted in the synthesis of predominantly short-chain ($C_{4:0}$ and $C_{6:0}$) and long-chain ($C_{14:0}$ and $C_{16:0}$) fatty acids (Table 1). This system was therefore chosen as the control. The addition of Fraction II did not increase the proportion of medium-chain acids synthesised though the rate of synthesis increased. Though there was a slight increase when Fraction III was added, Fraction IV caused

Table 2. The effect of Fraction IV on the pattern of fatty acids synthesised from [1-^{14}C]acetyl-CoA by fatty acid synthetase in the presence of the microsomal fraction

Addition	Lipid analysed*	Mole % fatty acids synthesised							Total nmole acetyl-CoA incorporated
		4:0	6:0	8:0	10:0	12:0	14:0	16:0	
None	TG	12	5	1	6	6	24	46	14
	TFA	31	6	1	4	4	18	36	34
Fraction IV	TG	10	5	15	40	16	9	5	17
	TFA	20	8	15	31	13	9	4	33

*TG = triglyceride; TFA = total fatty acids.
The incubation system (1.0 ml) contained 100 mM potassium phosphate buffer, pH 7.0, 8 mM $MgCl_2$, 3 mM potassium citrate, 10 mM $NaHCO_3$, 5 mM ATP, 0.24 mM NADPH, 1 mM EDTA, 5 mM rac-glycerol 3-phosphate, 40 µM [1-^{14}C]acetyl-CoA (4.2 µCi/µmole), 0.18 mg purified fatty acid synthetase and 2.5 mg microsomal protein from lactating rabbit mammary gland. Fraction IV (0.94 mg protein) was added as shown. Incubations were for 15 minutes at 37°C.

the pattern of fatty acids synthesised to completely alter from long-chain to medium-chain fatty acids and there was a concurrent decrease in the proportion of $C_{4:0}$ and $C_{6:0}$ acids in the triglycerides. The pattern of synthesised fatty acids in the triglyceride fraction in the presence of Fraction IV corresponds closely to that synthesised by the gland *in vivo* and *in vitro*. The rates of synthesis of the total fatty acids and of fatty acids in the triglyceride fraction were not changed by adding Fraction IV. The effects of Fraction IV were not due to the presence of unspecific proteins since they could not be brought about by bovine serum albumin (Table 1).

To obtain a more defined test system, purified fatty acid synthetase and the microsomal fraction from lactating rabbit mammary gland were used. It was then necessary to use [1-^{14}C]acetyl-CoA rather than [1-^{14}C]acetate. The microsomal fraction contained sufficient acetyl-CoA carboxylase (EC 6.4.1.2) activity (7.8 nmole CO_2 incorporated/min per mg protein) to supply malonyl-CoA

Table 3. The effects of trypsin and of heat treatment on Fraction IV

Treatment of Fraction IV	Lipid analysed*	Mole % fatty acids synthesised							Total nmole acetyl-CoA incorporated
		4:0	6:0	8:0	10:0	12:0	14:0	16:0	
Trypsin followed	TG	34	11	1	1	3	34	16	11
by inhibitor	TFA	48	8	1	1	2	25	15	27
Trypsin	TG	22	10	14	33	12	6	3	13
inhibitor	TFA	39	9	10	22	11	6	3	25
Supernatant after	TG	37	13	1	1	2	28	18	14
heat treatment	TFA	31	8	1	1	2	34	23	31
Sediment after	TG	38	14	1	1	2	31	13	13
heat treatment	TFA	41	12	1	1	2	25	18	32

*TG = triglycerides; TFA = total fatty acids.
Fraction IV (0.72 mg protein; 0.35 ml) was treated with 0.1 mg trypsin for 20 minutes at 37°C in 0.1 M potassium phosphate buffer, pH 7.0 and then with 0.5 mg of trypsin inhibitor for 20 minutes at 37°C. As a control, Fraction IV was incubated for 20 minutes at 37°C in 0.1 M potassium phosphate buffer, pH 7.0 and then with trypsin inhibitor for 20 minutes at 37°C. A further portion of Fraction IV (2.0 mg protein; 1.0 ml) was heated at 80°C for 10 minutes. After centrifugation, the sediment was resuspended in 1.0 ml of 0.1 M potassium phosphate buffer, pH 7.0. The resuspended sediment and the supernatant were tested for activity. 0.15 ml portions of the treated Fraction IV were incubated with purified fatty acid synthetase (0.124 mg) and 0.81 mg microsomal protein from lactating rabbit mammary gland. The incubation conditions are described in Table 2.

from the [1-^{14}C]acetyl-CoA. The results in Table 2 show that Fraction IV from the Sephadex column could also increase the proportion of medium-chain acids synthesised by this defined test system without altering the rate of fatty acid synthesis. This increase occurred in both the total fatty acids and in the triglyceride fraction.

These effects using this defined system were abolished when Fraction IV was pretreated with trypsin or preheated at 80°C for 10 minutes (Table 3). This indicates that the factor contains protein which is necessary for the effect.

From its elution pattern on Sephadex G-100 the molecular weight of the factor is in the range 40,000-50,000 though Fraction IV is contaminated with

haemoglobin (molecular weight 68,000). Additional evidence for the molecular weight range is that the factor does not pass through Amicon XM 50 ultrafiltration membranes which retain molecules with molecular weight in excess of 50,000.

Work is in progress to purify the factor further. It will be of interest to see whether the purified factor also contains carbohydrate or lipid.

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