Medium-chain fatty acid synthesis by goat mammary-gland fatty acid synthetase

The effect of limited proteolysis

Inger GRUNNET and Jens KNUDSEN

Institute of Biochemistry, Odense University, Campusvej 55, DK-5230 Odense, Denmark

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Fatty acid synthetase from goat mammary gland was subjected to limited proteolysis by trypsin and elastase. Both proteolytic enzymes selectively cleaved the chain-terminating thioester hydrolase component from the enzyme complex, leaving all other partial activities intact in the core peptides. Trypsin, but not elastase, caused extensive degradation of the released thioester hydrolase. The released thioester hydrolase could be purified to homogeneity by gel filtration. The molecular weight was estimated as 29000 and the enzyme showed only significant hydrolytic activity toward long-chain acyl-CoA esters. The core peptides retained the ability to synthesize medium-chain acyl-CoA esters in the presence of 2,6-di-*O*-methyl- α -cyclodextrin. The results conclusively show that the terminating thioester hydrolase of goat mammary-gland fatty acid synthetase is not involved in termination of medium-chain-length fatty acid synthesis by this enzyme.

Ruminant, rat, rabbit and mouse milk fat contain high proportions of medium-chain ($C_{8:0}$ – $C_{12:0}$) fatty acids (Breckenridge & Kuksis, 1967; Smith *et al.*, 1968), which have been shown to be synthesized within the mammary gland (Annison *et al.*, 1967; Carey & Dils, 1972). Termination of fatty acid synthesis at these chain lengths in rat, rabbit and mouse mammary gland is due to a specific medium-chain-terminating thioester hydrolase in the tissue (Knudsen *et al.*, 1976; Libertini & Smith, 1978; Smith & Stern, 1981). Ruminant mammary tissue does not contain a separate medium-chain-terminating thioester hydrolase; the fatty acid synthetase complex in this tissue is able to synthesize medium-chain fatty acids if the enzyme is incubated in the presence of the microsomal fraction (Grunnet & Knudsen, 1979). The medium-chain fatty acids synthesized by goat mammary-gland fatty acid synthetase in the presence of the microsomal fraction are directly incorporated into triacylglycerols without the need of an activation step (Grunnet & Knudsen, 1981). Bovine serum albumin, β -lactoglobulin and 2,6-di-*O*-methyl- α -cyclodextrin are also able to induce medium chain fatty acid synthesis by goat mammary fatty acid synthetase (Knudsen & Grunnet, 1982). The medium-chain fatty acids synthesized in the presence of 2,6-di-*O*-methyl- α -cyclodextrin are released from the synthetase complex by a transacylase reaction,

which catalyses their transfer from the enzyme complex to CoA. Beside this medium-chain transacylase activity, goat mammary synthetase also exhibits medium-chain thioester hydrolase activity (Grunnet & Knudsen, 1979), but it is not known if this thioester hydrolase activity is due to the long-chain-terminating thioester hydrolase activity of the synthetase or if it is a side reaction of the terminating medium-chain transacylase activity of the enzyme complex.

The terminating thioester component can be selectively released from various avian and mammalian fatty acid synthetases by limited proteolysis with trypsin, chymotrypsin or elastase (Smith *et al.*, 1976; Bedford *et al.*, 1978; Guy *et al.*, 1978; Puri & Porter, 1981; Rabinowitz *et al.*, 1982). We have investigated the effect of limited proteolysis of goat mammary-gland fatty acid synthetase complex on long- and medium-chain fatty acid synthesis, and on thioester hydrolase and medium-chain transacylase activities.

Materials and methods

Materials

Goats of mixed breeds at 14 days *post partum* were used. CoA and trypsin (bovine pancreas, 3.5 units/mg) were obtained from Boehringer, Mannheim, West Germany, and [1- 14 C]acetic anhydride

was from The Radiochemical Centre, Amersham, Bucks., U.K. Dithiothreitol and α -cyclodextrin were from Sigma Chemical Co., St. Louis, MO, U.S.A. Elastase (pig pancreas, 70 units/mg) and all other reagents were of analytical purity and were obtained from E. Merck, Darmstadt, West Germany. [$1\text{-}^{14}\text{C}$]Acetyl-CoA was synthesized as described by Stadtman (1957) and malonyl-CoA was synthesized by the method of Eggere & Lynen (1962). α -Cyclodextrin was methylated with dimethyl sulphate in a 1:1 (w/w) mixture of BaO and Ba(OH)₂ to give 2,6-di-*O*-methyl- α -cyclodextrin (Kuhn & Trischmann, 1963; Casu *et al.*, 1968).

Preparation of enzymes

Fatty acid synthetase was purified and assayed as described by Knudsen (1972). Medium-chain-terminating thioester hydrolase from rabbit mammary gland was purified as described by Knudsen *et al.* (1976), except that the preparative electrophoresis step was omitted.

Limited proteolysis of goat mammary fatty acid synthetase with trypsin and elastase

Fatty acid synthetase was incubated at 37°C in 0.1 M-potassium phosphate buffer (pH 7.0)/1.0 mM-EDTA/1.0 mM-dithiothreitol with 0.5 μg and 5 μg of trypsin and elastase/mg of synthetase respectively.

Assay of thioesterase activity

This was measured by a radiochemical assay. The system contained 0.05 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 8.0, 5 μM -1- ^{14}C -labelled acyl-CoA substrates (sp. radioactivity 1–5 Ci/mol) and enzyme (0.1–5 μg) in a final volume of 0.5 ml. Enzyme and buffer were pre-incubated for 5 min, and the reaction was started by addition of substrate, continued at 30°C for 1–2 min and stopped by addition of 2 ml of Dole's (1956) reagent.

Unesterified ^{14}C -labelled fatty acids were extracted by the method of Bar-Tana *et al.* (1971). The top phase, which contained the unesterified ^{14}C -labelled fatty acids, was evaporated to dryness; the fatty acids were dissolved in 10 ml of xylene containing 4.0 g of 2,5-diphenyloxazole/litre and the radioactivity was determined by liquid-scintillation counting. When butyryl-CoA and hexanoyl-CoA were used as substrates, the reaction was stopped by adding 0.5 ml of 0.1 M-HCl. The unesterified fatty acids were extracted with 4 $\times$ 1.0 ml of diethyl ether. A portion of the diethyl ether extract was counted for radioactivity as described above, except that the evaporation step was omitted.

Polyacrylamide-gel electrophoresis and molecular-weight determination

The polyacrylamide-gel-electrophoresis method used was essentially that used by Laemmli (1970)

modified as described by Grunnet & Knudsen (1978b). The molecular-weight markers used were bovine serum albumin (mol.wt. 68 000), ovalbumin (mol.wt. 43 000), carbonic anhydrase (mol.wt. 29 000), soya-bean trypsin inhibitor (mol.wt. 21 700) and cytochrome *c* (mol.wt. 11 700).

Purification of the thioester hydrolase released from goat mammary-gland fatty acid synthetase with elastase

Goat mammary-gland fatty acid synthetase (10–100 mg) after incubation with elastase for 3 h was loaded on a column (2.5 cm $\times$ 55 cm) of Sephadex G-75. The column was eluted with 10 mM-potassium phosphate buffer, pH 7.0, containing 1.0 mM-EDTA and 0.5 mM-dithiothreitol (at 25 ml/h), and fractions (6.4 ml) containing the enzyme were pooled and stored at -80°C .

Protein determination

Proteins were precipitated with 15% (w/v) trichloroacetic acid and determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Determination of fatty acids synthesized

Fatty acid synthetase was incubated at 37°C in 100 mM-potassium phosphate buffer, pH 7.0, which contained 40 μM -[1- ^{14}C]acetyl-CoA, 1.0 mM-EDTA and 240 μM -NADPH. Malonyl-CoA was infused continuously during the incubation by using a model 971 Harvard infusion pump fitted with eight 1.0 ml syringes. The infusion tubes were fixed near the bottom of the incubation vessels, which were shaken in a rotary motion in a Buchler Evapomix apparatus at 37°C. Fatty acid synthetase and 2,6-di-*O*-methyl- α -cyclodextrin were added as indicated. The incubation was stopped by adding NaOH (5 M) (1 ml/ml of incubation). Total fatty acids synthesized were measured and the pattern of fatty acids synthesized was determined by radio-g.l.c. (Knudsen *et al.*, 1981).

Assay of decanoyl transacylase activity

The rate of transfer of decanoate from its CoA ester to pantetheine was determined as follows. The reaction mixture (0.25 ml) contained 0.166 mM-[1- ^{14}C]decanoyl-CoA (sp. radioactivity 1.15–5.0 Ci/mol), 3.4 mM-pantetheine, 0.2 mM-potassium phosphate buffer, pH 6.6, and 1.0 mM-EDTA. Enzyme (1–2 μg), decanoyl-CoA and buffer were pre-incubated for 10 min at 30°C, and the reaction was started by addition of pantetheine. After 1 min the reaction was stopped by addition of 25 μl of 70% (v/v) HClO₄. Decanoyl-pantetheine and non-esterified fatty acids were extracted with 3 $\times$ 0.50 ml of diethyl ether. The diethyl ether layer was removed and evaporated to dryness. The decanoyl-pant-

etheine esters were dissolved in 4 ml of xylene containing 4 g of 2,5-diphenyloxazole/litre and the radioactivity was determined by liquid-scintillation counting. Decanoyl-pantetheine was identified by high-performance liquid chromatography on a stainless-steel column (25 cm $\times$ 0.41 cm) packed with Lichrosorb RP18 (10 μ m). The eluent consisted of a mixture of methanol and water; the flow rate was 1.7 ml/min. The elution was initiated at 60% (v/v) methanol with a gradient of 0.2% methanol/min to 64% methanol. The eluate from the column was collected and the radioactivity in the fractions was determined. Decanoyl-pantetheine was identified by running an authentic standard of decanoyl-pantetheine, and measuring the A_{233} .

Results

Effect of elastase and trypsin on goat mammary fatty acid synthetase

Treatment of goat mammary fatty acid synthetase with elastase or trypsin resulted in a time-dependent inhibition of synthetase activity (Fig. 1). The residual fatty acid synthetase activities after 120 min incubation with elastase and trypsin were 5% and less than 1% of initial activity respectively.

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate showed that the cleavage of thioester hydrolase from the synthetase complex was much more clean with elastase than with trypsin. When elastase was used, a clear thioester hydrolase band with the same mobility as carbonic anhydrase appeared with time, with no evidence of further degradation (Figs. 2a and 2b). If trypsin was used, a much weaker thioester hydrolase

band appeared with time, and the appearance of smaller peptides was observed (results not shown). This indicates further 'nicking' of the released thioester hydrolase when trypsin was used to release this enzyme from the synthetase complex.

Fig. 1. *Effect of trypsin or elastase on goat mammary-gland fatty acid synthetase activity*

For experimental details, see the Materials and methods section. Fatty acid synthetase (5.2 mg/ml) was incubated with trypsin (▲) or elastase (○), or without added proteolytic enzymes (●). Bars show $\pm$ half the difference between two individual incubations; where no bars are shown, the deviations are within the range of the symbols. The values shown are typical results from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Fig. 2. *Polyacrylamide-gel electrophoresis on 5% gels in the presence of sodium dodecyl sulphate of trypsin- or elastase-treated goat mammary fatty acid synthetase*

For experimental details, see the Materials and methods section. Fatty acid synthetase (1.31 mg/ml) was incubated with trypsin (a) or elastase (b). Tracks: (a) 1 and 2, molecular-weight markers [a, bovine serum albumin (mol.wt. 68000), b, ovalbumin (mol.wt. 43000), c, carbonic anhydrase (mol.wt. 29000), d, myosin (mol.wt. 220000), e, α -macroglobulin (mol.wt. 185000), f, phosphorylase a (mol.wt. 94000)]; 3, 4 and 5, 120 min, 30 min and 5 min trypsin treatment respectively; 6, control treated enzyme; (b), as (a), except that elastase was used instead of trypsin. The patterns shown are typical results from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Similar effects of trypsin on the thioester hydrolase component have been observed in experiments with rat mammary fatty acid synthetase (Dileepan *et al.*, 1978). Attempts to purify the thioesterase component after incubations with trypsin showed that very little or no thioester hydrolase could be recovered from these incubations.

Purification and molecular weight of thioester hydrolase released from goat mammary fatty acid synthetase with elastase

The thioester hydrolase could be obtained in homogenous form by chromatographing the incubation mixture from elastase treatment of fatty acid synthetase on Sephadex G-75 (Fig. 3). Attempts to separate the proteins in the void-volume peak from the Sephadex G-75 column (Fig. 3) into the separate peptides that had been shown to be present by polyacrylamide-gel electrophoresis (Fig. 2) were unsuccessful. The proteins in this peak were eluted as a single peak on Sepharose 6B with a molecular weight of about 400 000 (results not shown).

The purified thioesterase component released from the synthetase was homogenous on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Fig. 4). The mobility of the thioester hydrolase released was identical with that of carbonic anhydrase, mol.wt. 29 000 (Fig. 4). This is slightly lower than that of the thioester hydrolase component released from fatty acid synthetase of rat mammary gland (mol.wt. 32 000) (Smith *et al.*, 1976), pigeon liver (mol.wt. 36 000)

Fig. 3. Sephadex G-75 gel filtration of elastase-treated goat mammary fatty acid synthetase

For experimental details, see the Materials and methods section. Fatty acid synthetase (42 mg) was incubated with elastase for 240 min. The gel-filtration pattern shown is a typical result from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Fig. 4. Polyacrylamide-gel electrophoresis on 15% gels in the presence of sodium dodecyl sulphate of purified thioester hydrolase released from goat mammary synthetase with elastase

For experimental details, see the Materials and methods section. Lanes: 1, molecular-weight markers [a, bovine serum albumin (mol.wt. 68 000), b, ovalbumin (mol.wt. 43 000), c, carbonic anhydrase (mol.wt. 29 000), d, soya-bean trypsin inhibitor (mol.wt. 21 700) and e, cytochrome c (mol.wt. 11 700)]; 2, thioester hydrolase. The pattern shown is a typical result from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

(Puri & Porter, 1981) and goose uropygial gland (mol.wt. 33 000) (Bedford *et al.*, 1978).

Re-activation of the thioester hydrolase-stripped fatty acid synthetase, and immunological properties of this enzyme and the released thioester hydrolase

Goat mammary gland fatty acid synthetase stripped of thioester hydrolase could be re-activated with medium-chain-terminating thioester hydrolase isolated from rabbit mammary gland (Table 1), and a linear increase in activity was obtained with increasing amounts of thioester hydrolase added. By contrast, thioester hydrolase released from goat synthetase with elastase was unable to re-activate the stripped synthetase. In experiments with trypsin treatment of fatty acid synthetase from rat mammary gland (Libertini & Smith, 1978), the medium-chain-terminating thioester hydrolase from this tissue could also re-activate stripped synthetase. Elastase-treated rabbit mammary-gland fatty acid synthetase could also be re-activated with the medium-chain-terminating thioester hydrolase from this tissue (Guy *et al.*, 1978). Two 65 000-dalton thioester hydrolases isolated from rabbit mammary

Table 1. *Re-activation of goat mammary-gland fatty acid synthetase stripped of thioester hydrolase*
Fatty acid synthetase activity was assayed as described in the Materials and methods section. Stripped fatty acid synthetase was incubated in 0.5ml of 0.1M-potassium phosphate buffer, pH 7.0, containing 1mM-EDTA. Terminating medium-chain acylthioester hydrolase was added as indicated. The values for NADPH oxidized are means $\pm$ half the difference between duplicates. The values shown are typical results from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Fatty acid synthetase	Rabbit mammary-gland medium-chain thioester hydrolase added (μ g)	NADPH oxidized (nmol/min per mg of protein)
Control incubated	—	797 $\pm$ 7
Stripped		
50 μ g	0	72 $\pm$ 0
50 μ g	26	143 $\pm$ 2
50 μ g	53	206 $\pm$ 1
50 μ g	106	316 $\pm$ 2
100 μ g	106	295 $\pm$ 2

Fig. 5. *Immunodiffusion studies*
The centre well contained anti-(fatty acid synthetase) γ -globulin. Outer wells: 1 and 4, goat mammary fatty acid synthetase; 2 and 5, released thioester hydrolase from goat mammary synthetase; 3 and 6, goat mammary synthetase stripped of thioester hydrolase. For experimental details, see the Materials and methods section.

Fig. 6. *Substrate specificity of the thioesterase component released from goat mammary-gland fatty acid synthetase with elastase*
Incubation conditions are described in the Materials and methods section. Thioesterase (0.2 μ g) was incubated at 30°C for 1.0min. O, Palmitoyl-CoA; ●, myristoyl-CoA. The values shown are typical results from at least three individual fatty acid synthetase preparations. Bars show $\pm$ s.d. for triplicate incubations; where no bars are shown the s.d. is within the range of the symbols.

gland and rat liver were unable to re-activate the stripped goat synthetase (results not shown). Both the released thioester hydrolase and the stripped synthetase were recognized by anti-(fatty acid synthetase) γ -globulins, giving strong precipitation lines in double-diffusion studies (Fig. 5). Similar results were obtained in experiments with trypsin treatment of fatty acid synthetase from rat

mammary gland (Smith *et al.*, 1976) and goose uropygial gland (Bedford *et al.*, 1978), although they obtained spurs between the released thioester hydrolase and native fatty acid synthetase in double-diffusion studies.
Specificity of the thioester hydrolase released from goat mammary fatty acid synthetase
The released thioester hydrolase hydrolysed myristoyl-CoA and palmitoyl-CoA (Fig. 6), the

activity toward myristoyl-CoA being about 3% of that obtained with palmitoyl-CoA. The released thioester hydrolase showed very little or no activity towards myristoyl-CoA and shorter-chain acyl-CoA model substrates at the substrate concentrations used (5 μ M).

Effect of elastase on the medium- and long-chain thioester hydrolase and decanoyl transacylase activities exhibited by goat mammary fatty acid synthetase

Incubation of fatty acid synthetase with elastase for 240 min decreased fatty acid synthetase activity to 9.1% of that in control incubations, and decreased hydrolytic activity toward palmitoyl-CoA to 4.5% of control enzyme (Table 2). Hydrolytic activity towards decanoyl-CoA and decanoyl transacylase activity were not significantly affected by elastase treatment.

Fatty acid synthesized by thioester hydrolase-stripped goat mammary fatty acid synthetase in the absence and presence of 2,6-di-O-methyl- α -cyclodextrin

Stripping the thioester hydrolase component from goat mammary synthetase changed the pattern of fatty acids synthesized. Myristic acid was the main long-chain fatty acid synthesized by the control incubated enzyme under the incubation conditions used (Table 3). Stripping off the thioester hydrolase resulted in a decreased rate of long-chain fatty acid synthesis and a change in pattern of long-chain fatty acids synthesized, from mainly myristic acid to exclusively palmitic acid and stearic acid. The amounts of palmitic acid plus stearic acid synthesized are 0.24 and 0.18 nmol for the trypsin- and elastase-treated enzyme respectively, which is less than 1 mol per mol of fatty acid synthetase. The incubation mixture contained 0.32 and 0.34 nmol of

Table 2. *Effect of elastase on fatty acid synthetase, acylthioester hydrolase and decanoyl-transacylase activities in goat mammary-gland fatty acid synthetase*

Enzyme activities were determined as described in the Materials and methods section. Values are means $\pm$ half the difference between duplicates. The values shown are typical results from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Fatty acid synthetase	NADPH oxidized (nmol/min per mg of protein)	Acyl-CoA esters hydrolysed (nmol/min per mg of protein)		Decanoyl-panthetheine synthesized (nmol/min per mg of protein)
		Decanoyl-CoA	Palmitoyl-CoA	
Control incubated	797 $\pm$ 7	3.3 $\pm$ 2.0	200 $\pm$ 0	3589 $\pm$ 89
Elastase treated	72 $\pm$ 0	3.2 $\pm$ 0.7	9 $\pm$ 0	3157 $\pm$ 332

Table 3. *Fatty acids synthesized by goat mammary-gland fatty acid synthetase stripped of thioester hydrolase*

The incubation system is described in the Materials and methods section. The incubations contained control incubated fatty acid synthetase (0.13 mg), elastase-treated synthetase (0.143 mg) or trypsin-treated synthetase (0.137 mg), and 40 μ M-[1- 14 C]acetyl-CoA (sp. radioactivity 3.25 Ci/mol) in a total volume of 0.5 ml. Malonyl-CoA was infused at a rate of 1.09 nmol/min. 2,6-Di-O-methyl- α -cyclodextrin was added as indicated. The values for radioactivity distribution in fatty acids are means of two determinations, and those for total acetate incorporation are means $\pm$ half the difference between duplicates. The values shown are typical results from at least three individual experiments performed with at least two different fatty acid synthetase preparations.

Enzyme	2,6-Di-O-methyl- α -cyclodextrin added (mg)	Distribution of radioactivity in fatty acids (%)								Total acetate incorporation (nmol)
		C _{4:0}	C _{6:0}	C _{8:0}	C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	
Control treated	0	52	10	2	4	7	20	5	—	2.53 $\pm$ 0.22
	1.2	39	19	11	19	9	3	—	—	3.71 $\pm$ 0.07
	2.4	43	18	17	19	2	1	—	—	4.48 $\pm$ 0.03
Trypsin treated	0	75	9	3	—	—	—	6	7	1.87 $\pm$ 0.17
	1.2	58	15	9	12	4	2	—	—	3.77 $\pm$ 0.13
	2.4	50	25	9	15	1	—	—	—	3.71 $\pm$ 0.08
Elastase treated	0	84	7	—	—	—	—	4	5	2.05 $\pm$ 0.01
	1.2	48	15	12	20	4	1	—	—	4.49 $\pm$ 0.24
	2.4	48	19	14	19	—	—	—	—	4.46 $\pm$ 0.13

synthetase respectively. Trypsin treatment of rat mammary fatty acid synthetase resulted in a similar change in pattern of long-chain fatty acids synthesized (Smith & Libertini, 1979). The long-chain fatty acids synthesized in their experiments remained bound to the fatty acid synthetase complex, owing to the lack of thioester hydrolase activity (Smith & Libertini, 1979). It is therefore reasonable to assume that the long-chain fatty acids synthesized in the present experiment are enzyme-bound, and cannot be released from the proteinase-treated enzyme. Addition of 2,6-di-*O*-methyl- α -cyclodextrin restored the rate of fatty acid synthesis by the proteinase-treated enzyme and induced medium-chain fatty acid synthesis to almost the same extent as in control incubated enzyme.

These experiments clearly show that the medium-chain-terminating transacylase is a different enzyme from the long-chain terminating thioester hydrolase.

Discussion

The thioester hydrolase component of goat mammary-gland fatty acid synthetase could be selectively cleaved from the synthetase complex by both trypsin and elastase. The decrease in fatty acid synthetase activity was proportional to the decrease in palmitoyl thioester hydrolase activity (Table 1). Both elastase-treated synthetase (Table 2) and trypsin-treated synthetase (results not shown) could be re-activated by addition of medium-chain-terminating thioester hydrolase from rabbit mammary gland. Both proteolytically treated enzymes were also able to synthesize medium-chain fatty acids in the presence of 2,6-di-*O*-methyl- α -cyclodextrin (Table 3). This shows that all other partial activities are intact in the proteolytically treated synthetase complex.

The re-activation results agree with those obtained with limited proteolysis of fatty acid synthetase from rat mammary gland (Libertini & Smith, 1978) and rabbit mammary gland (Guy *et al.*, 1978).

The present results show that elastase is superior to trypsin in cleaving intact thioester hydrolase from fatty acid synthetase. Very little thioester hydrolase protein could be observed on polyacrylamide gels (Fig. 2), or was obtained after Sephadex G-75 chromatography of the incubation mixture after trypsin treatment.

The thioester hydrolase released from goat mammary fatty acid synthetase has a slightly lower molecular weight (29000) than the thioester hydrolase component released from other mammalian and avian fatty acid synthetases (32000–36000). The ability of the released thioester hydrolase only to hydrolyse long-chain acyl-CoA esters is similar to the specificity of the hydrolase released from rat

mammary gland and liver (Lin & Smith, 1978) and uropygial gland (Bedford *et al.*, 1978).

However, the specificity of the thioester hydrolase released from goat mammary synthetase complex does not agree with the results obtained with the hydrolase component from cow mammary-gland synthetase (Abinejad *et al.*, 1981). At 5 μ M-butyryl-CoA the thioester hydrolase released from goat synthetase barely shows hydrolytic activity towards this substrate. When measured at 22.5 μ M- and 45.0 μ M-butyryl-CoA the hydrolytic activity was less than 3% and 4% respectively of that towards palmitoyl-CoA at 5 μ M (results not shown). The hydrolase component from cow enzyme hydrolysed butyryl-CoA at a rate of 27% of that with palmitoyl-CoA (Abinejad *et al.*, 1981). We find this surprising, since both cow and goat mammary gland synthesize butyric acid *in vivo*. We do not have any explanation for this difference.

The present results show that it is unlikely that the thioester hydrolase component of the goat mammary-gland fatty acid synthetase is involved in the termination of short- and medium-chain fatty acids because it cannot hydrolyse short- and medium-chain acyl thioesters.

The medium-chain acylthioester hydrolase activity of the untreated goat mammary synthetase complex (Grunnet & Knudsen, 1978a) is still present in the elastase-treated enzyme complex (Table 2). The partial activity in the enzyme complex responsible for the medium-chain thioester hydrolase activity is unknown, but one possibility could be that it is a side reaction of the medium-chain transacylase.

The medium-chain thioester hydrolase activities present in treated and untreated goat mammary synthetase were identical, but were very low compared with the decanoyl transacylase activity. The amounts of non-esterified fatty acids released in the decanoyl transacylase assay (150 μ M-decanoyl-CoA) amounted to less than 3% of the decanoyl-pantetheine synthesized by native and elastase-treated synthetase (results not shown). The very low decanoyl-thioester hydrolase activity is unable to compete with the elongation reaction, which explains why the goat mammary synthetase only synthesized very small amounts of non-esterified medium-chain fatty acids even at rate-limiting malonyl-CoA concentrations in the presence and absence of 2,6-di-*O*-methyl- α -cyclodextrin (Knudsen & Grunnet, 1982).

The results in Table 3 show that both trypsin- and elastase-treated goat mammary synthetase have retained full ability to synthesize medium- and short-chain fatty acids (as acyl-CoA esters) in the presence of 2,6-di-*O*-methyl- α -cyclodextrin. The goat synthetase complex also retains its ability to catalyse the transfer of decanoate from CoA to

pantetheine after elastase treatment (Table 2). We have previously shown that short-chain fatty acids synthesized by cow mammary-gland synthetase (Hansen & Knudsen, 1980) and medium-chain fatty acids synthesized in the presence of the methylated α -cyclodextrin by goat mammary synthetase are almost exclusively released as acyl-CoA esters (Knudsen & Grunnet, 1982).

The present results therefore further support the suggestion that medium-chain fatty acid synthesis by goat mammary fatty acid synthetase is terminated by a transacylase (Knudsen & Grunnet, 1982), and show that this enzyme is different from the long-chain thioester hydrolase. The question arises whether the decanoyltransferase is identical with the loading transacylase, since we have shown that goat and cow mammary-gland fatty acid synthetase both use decanoyl-CoA effectively as primer (Knudsen & Grunnet, 1980). The final clarification of this problem will have to await the determination of the primary amino acid sequence of the decanoyl and acetyl transacylase components of goat mammary synthetase.

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