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MEDIUM CHAIN ACYL-THIOESTER HYDROLASE ACTIVITY IN GOAT AND RABBIT MAMMARY GLAND FATTY ACID SYNTHETASE COMPLEXES

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Summary: The goat mammary gland fatty acid synthetase hydrolysed both medium ($C_{8:0}$, $C_{10:0}$) and long ($C_{16:0}$, $C_{18:0}$) chain length acyl CoA esters, whereas the enzyme from rabbit mammary gland only hydrolysed long chain length acyl CoA esters. The medium chain acyl-thioester hydrolase activity of goat mammary gland fatty acid synthetase was much less sensitive to inhibition by phenylmethanesulfonyl-fluorid than the long chain acyl-thioester hydrolase activity. These results indicate the presence of either two acyl-thioester hydrolases with different specificity or one acyl-thioester hydrolase containing two different active sites.

It has been reported that pigeon liver fatty acid synthetase can be separated into two half molecular weight subunits, each containing acyl-thioester hydrolase activity (1). Further indications for the presence of two acyl-thioester hydrolases in pigeon liver fatty acid synthetase were given in inhibitor studies with PMSF (2). It was shown that the acyl-thioester hydrolase in this enzyme complex could be specifically inhibited by PMSF, and that each enzyme complex bound about two moles PMSF per moles fatty acid synthetase (500.000 molecular weight).

In the present study we have investigated the specificity of the acyl-thioester hydrolases of fatty acid synthetase complexes from goat and rabbit mammary gland in the presence and absence of PMSF.

The results show that the goat mammary gland fatty acid synthetase hydrolyses both medium and long chain length acyl-

ABBREVIATION : PMSF: phenylmethylsulfonyl-fluorid

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CoA model substrates, whereas the rabbit enzyme only hydrolyses the long chain length acyl-CoA esters. Furthermore it was shown that the long chain length acyl-thioester hydrolase activity of goat mammary gland was much more sensitive to PMSF inhibition than the medium chain length acyl-thioester hydrolase activity. This may suggest that goat mammary gland fatty acid synthetase contains two different acyl-thioester hydrolases.

MATERIALS AND METHODS

Dithiothreitol and PMSF was obtained from Sigma Chemical Co. St. Louis, Mo., U.S.A. All other reagents used were of analytical purity. [1-^{14}C] acyl-CoA esters were synthesized as described by J. Knudsen *et al* (3). Fatty acid synthetase were purified according to the method of Knudsen (4). Lactating mammary gland of New Zealand white rabbits and goats mixed breed were both used at 14 days post partum.

PMSF treatment of the fatty acid synthetase was carried out in 4% isopropanol. Varying concentrations of PMSF in isopropanol was mixed with fatty acid synthetase (1-3 mg/ml) and incubated for 1 hr at 30°C. The enzyme was precipitated by adding 200 g solid $(NH_4)_2SO_4$ per l and then dialysed against 0.25 M phosphate buffer pH 7.0, 1 mM EDTA and 1 mM DTT for 2 hr. Control experiments showed that 4% isopropanol had no effect on the acyl-thioester hydrolase specificity.

Incubations were made as described by Knudsen *et al* (3) with following modifications. All acyl-CoA esters were used in 3 µM concentrations, and the rate of hydrolysis was determined by counting the radioactivity extracted into the top phase. Incubation time was 3 minutes in experiments with $C_{4:0}$ to $C_{14:0}$ acyl-CoA esters, and 1 min in experiments with $C_{16:0}$ and $C_{18:0}$ acyl-CoA esters. Less than 10% of the substrates was hydrolysed during the incubation period.

RESULTS AND DISCUSSION

The fatty acid synthetase from goat mammary gland showed significant hydrolytic activity towards both medium and long chain acyl-thioester model substrates whereas, in contrast, the rabbit enzyme hydrolysed only long chain acyl-thioesters (fig. 1A and B, hatched bars).

This indicates that goat mammary gland fatty acid synthetase contains either one acyl-thioester hydrolase with a specificity different from that of the rabbit enzyme, or contains two acyl-thioester hydrolases like it has been reported for pigeon liver fatty acid synthetase (1,2).

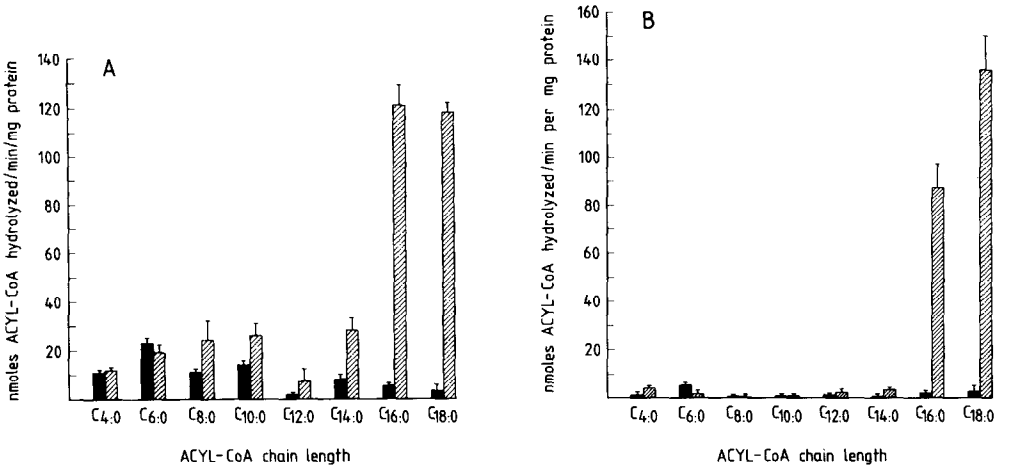

Fig. 1. The effect of PMSF on fatty acid synthetase acyl-thioester hydrolase activity. The fatty acid synthetases were treated with 1.0 mM PMSF (black bars) as described under methods, the control (hatched bars) contained 4% isopropanol. A. Goat mammary gland fatty acid synthetase. B. Rabbit mammary gland fatty acid synthetase. (S.D. shown).

Further indications for the presence of two different acyl-thioester hydrolases in the goat enzyme complex was obtained from inhibitor studies with PMSF. PMSF is a specific inhibitor of serine containing esterases, and 1 mM PMSF has been shown to completely inhibit the terminating acyl-thioester hydrolase of the fatty acid synthetase complex from pigeon liver (2). We found that 1 mM PMSF almost totally inhibited the long chain length acyl-thioester hydrolase activity of the goat fatty acid synthetase but inhibited the activity towards medium chain acyl-thioesters by only 50% (fig. 1A black bars). Incubated under same conditions all acyl-thioester hydrolase activity of rabbit fatty acid synthetase was strongly inhibited by 1 mM PMSF (fig. 1B black bars).

Furthermore the hydrolase activity of goat mammary gland fatty acid synthetase towards C_{16} acyl-CoA was much more sensitive to PMSF inhibition compared to its activity towards C_{10} acyl-CoA. Thus 100 μM PMSF resulted in a 50% inhibition of

activity towards C_{16} acyl-CoA but did not inhibit activity towards C_{10} acyl-CoA (fig. 2).

Kumar's (2) evidence for the presence of two acyl-thioester hydrolases per mole pigeon liver fatty acid synthetase was that two moles of PMSF per mole fatty acid synthetase were nescessary to inhibit the fatty acid synthetase completely. Like Kumar, we can not distinguish whether we have one enzyme with two active sites per mole fatty acid synthetase, or two enzymes with different sensitivity to PMSF. Lornitzo et al (1) showed that each of the two subunits of pigeon liver fatty acid synthetase contained an acyl-thioester hydrolase. It is therefore reasonable to suggest that goat mammary gland fatty acid synthetase contains two different acyl-thioester hydrolases which have different specificity towards acyl-CoA esters and different sensitivity to PMSF inhibition.

In this connection it is interesting to note that Barnes et al found two acyl-thioester hydrolases in E. Coli (5 & 6). One hydrolysed long chain length acyl-CoA esters and was sensitive to diisopropylfluorophosphate while the other hydrolysed both medium and long chain acyl-CoA esters, and was insensitive to diisopropylfluorophosphate.

The synthesis of medium chain length fatty acids in rabbit and rat mammary gland has been shown to be controlled by a specific chain length terminating acyl-thioester hydrolase (MW 29.000), which hydrolyses both medium and long chain length acyl-CoA model substrates (3 & 7). Goat mammary gland also synthesises large amounts of medium chain length fatty acids (decanoic acid) (8), but we have not been able to find a similar medium chain length fatty acid terminating enzyme in goat mammary gland cytosol (unpublished results). It is therefore

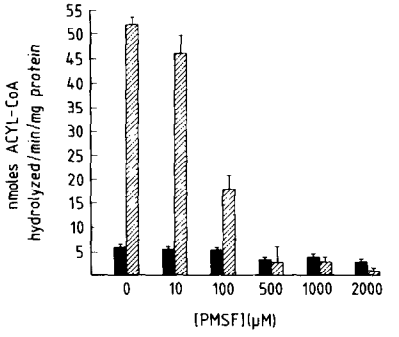

Fig. 2. The effect of various concentrations of PMSF on goat fatty acid synthetase acyl-thioester hydrolase activity. The enzyme was incubated with various concentrations of PMSF as described under methods. The activity towards $C_{10:0}$-acyl CoA (black bars) and $C_{16:0}$-acyl CoA (hatched bars) was measured (s.d. shown).

possible that the medium chain length acyl-thioester hydrolase activity of the goat mammary gland fatty acid synthetase complex could be involved in terminating fatty acid synthesis at C_{10} chain-length in goat mammary gland.

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