MAMMALIAN FATTY ACID SYNTHETASE: EVIDENCE FOR SUBUNIT IDENTITY AND SPECIFIC REMOVAL OF THE THIOESTERASE COMPONENT USING ELASTASE DIGESTION

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1. Introduction

Mammalian fatty acid synthetase is a multifunctional enzyme containing 7 enzyme activities and an acyl carrier function on only 2 polypeptide chains [1]. These 2 polypeptides both have mol. wt 250 000 and cannot be resolved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate [2–4]. However it has not been clear whether or not the subunits are identical.

In this paper we present information on the cleavage of rabbit mammary gland fatty acid synthetase by elastase, which is difficult to reconcile with the view that the subunits are different. If our view is correct, fatty acid synthetase represents a remarkable sample of a multifunctional polypeptide, with 7 activities and a prosthetic group on one polypeptide chain. We also show that the first cleavage by elastase specifically removes the thioesterase component. This causes inactivation of fatty acid synthetase despite the fact that all 7 partial enzyme activities are unimpaired.

2. Methods

2.1. *Materials*

Fatty acid synthetase was purified to homogeneity from lactating rabbit mammary gland [4].

[^{14}C]Palmityl-CoA was from the Radiochemical Centre, Amersham. Palmityl-CoA and decanoyl-CoA were from P.L. Biochemicals, Milwaukee, WI. Other biochemicals were from Sigma Chemical Co., Poole, Dorset.

2.2. *Enzyme assays*

Fatty acid synthetase was assayed spectrophotometrically according to [5], palmityl-CoA thioesterase by the procedure in [6], and decanoyl-CoA thioesterase according to [7].

2.3. *Partial purification of the medium-chain thioesterase*

A 90 000 × *g* supernatant was prepared from lactating rabbit mammary glands as in [4], and the protein precipitating between 40% and 60% saturated ammonium sulphate was dialysed against 100 mM sodium phosphate/1 mM EDTA/15 mM mercaptoethanol, pH 7.0. The solution was then subjected to gel filtration on a column of Sephadex G-100 Superfine (50 × 2 cm). Fractions containing decanoyl-CoA thioesterase activity, which eluted at a V/V_0 of around 2.0, were pooled. The preparation was completely free of fatty acid synthetase activity.

2.4. *Analytical methods*

Electrophoresis in 4% polyacrylamide gels in the presence of sodium dodecyl sulphate was carried out as in [4]. Amino-terminal analysis was by the microdansyl chloride method in [8].

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3. Results

3.1. *Inactivation and cleavage of fatty acid synthetase*

When fatty acid synthetase (1.0 mg/ml) was incubated with elastase (1 μg/ml) fatty acid synthetase activity was lost completely within 40 min (fig.1). This loss of activity correlated with a conversion of the 250 000 dalton subunit into fragments of 220 000 and 35 000 daltons (fig.2). Upon prolonged digestion of up to 8 h, the 220 000 fragment gradually disappeared and 2 new fragments of 120 000 and 110 000 daltons appeared (fig.2).

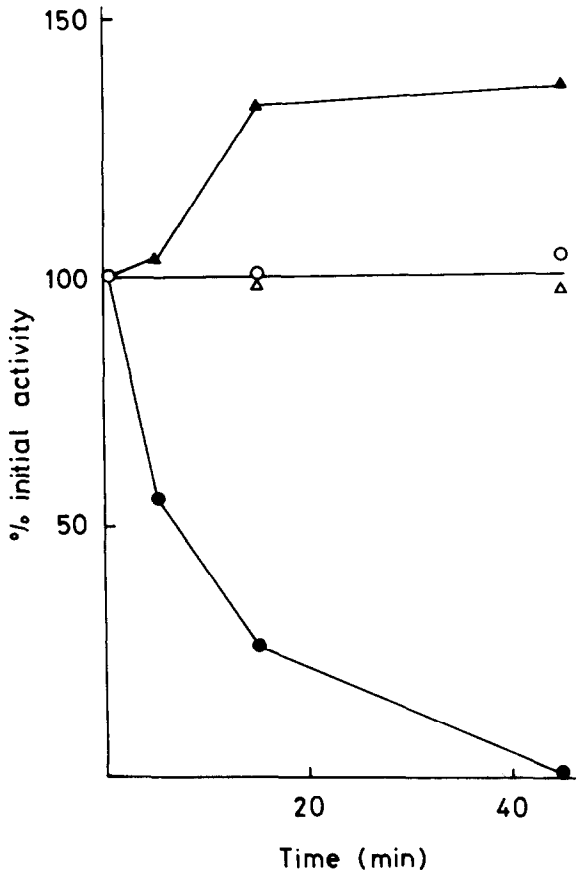

Fig.1. Effect of elastase on the activity of fatty acid synthetase and its thioesterase component. Fatty acid synthetase (1.0 mg/ml) was incubated with elastase (1.0 μg/ml) in 125 mM phosphate buffer, 0.5 mM EDTA, 15 mM mercaptoethanol, at 37°C. Aliquots were removed at intervals and assayed for fatty acid synthetase (•) and palmityl-CoA thioesterase (▲). Open symbols show the activities in a control incubation in which elastase was omitted.

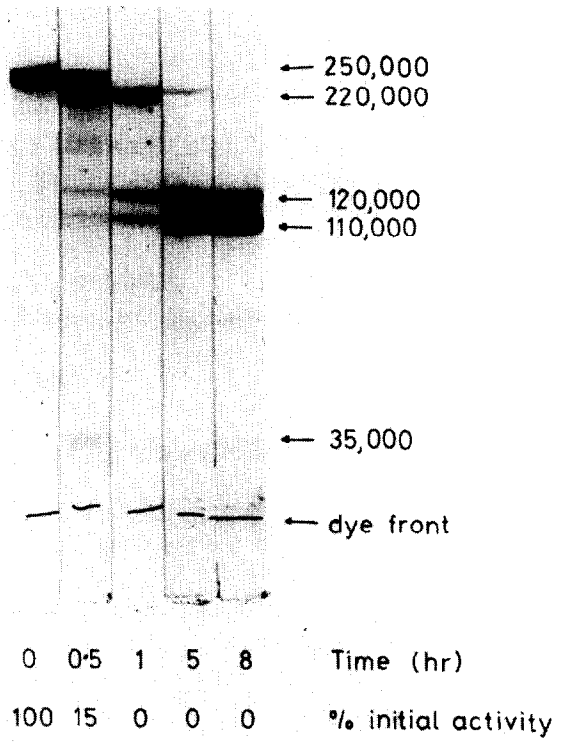

Fig.2. Cleavage of the fatty acid synthetase subunit with elastase. Fatty acid synthetase was incubated with elastase as described in fig.1 legend. At the times indicated aliquots were removed and analysed using 4% polyacrylamide gels in the presence of sodium dodecyl sulphate. The molecular weights of the various peptides were estimated using the marker proteins described in [4]. The percentage of initial fatty acid synthetase activity remaining at each time point is also shown.

3.2. *Demonstration that the 35 000 dalton fragment contains the thioesterase activity*

During the course of digestion with elastase the thioesterase activity of fatty acid synthetase, assayed using palmityl-CoA as substrate, initially increased by 40% (fig.1). If the enzyme was digested with elastase for 60 min and the solution was then brought to 45% saturation with ammonium sulphate and centrifuged, the supernatant contained 54% thioesterase activity and contained only the 35 000 dalton fragment as judged by electrophoresis on 4% (fig.3) and 10%

polyacrylamide gels in the presence of sodium dodecyl sulphate. This fraction contained a single amino-terminal, lysine, by the dansyl chloride method. We could not detect a free amino-terminal on the undigested enzyme, in agreement with [2] on the rat liver enzyme.

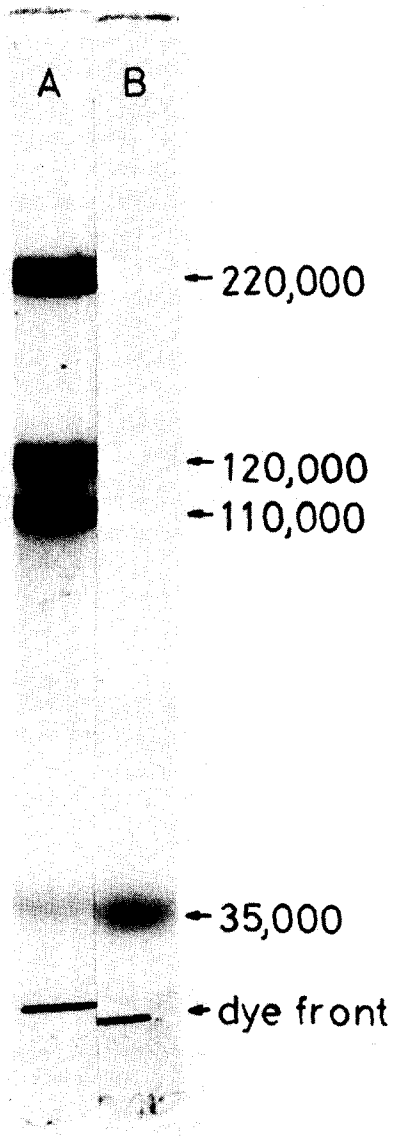

Fig.3. Purification of the thioesterase component. Fatty acid synthetase was digested for 90 min as described in fig.1 legend. The photograph shows 4% polyacrylamide gels run in the presence of sodium dodecyl sulphate of: (A) the total digest; (B) that fraction of the digest which did not precipitate in a 45% saturated solution of ammonium sulphate. Molecular weights were estimated using the marker proteins described in [4].

3.3. *Reactivation of elastase-inactivated fatty acid synthetase using medium-chain thioesterase*

Although elastase treatment did not reduce the thioesterase activity of fatty acid synthetase measured using palmityl-CoA as substrate, fatty acid synthetase was completely inactivated (fig.1). In order to test whether one of the other partial activities was destroyed, we partially purified the medium-chain thioesterase from lactating rabbit mammary gland. This is an enzyme found only in lactating mammary gland [7], which can modify the product specificity of fatty acid synthetase to produce C_8 and C_{10} as well as C_{16} fatty acids. When the medium-chain thioesterase was added to elastase-inactivated fatty acid synthetase, the fatty acid synthetase activity was reactivated completely (fig.4).

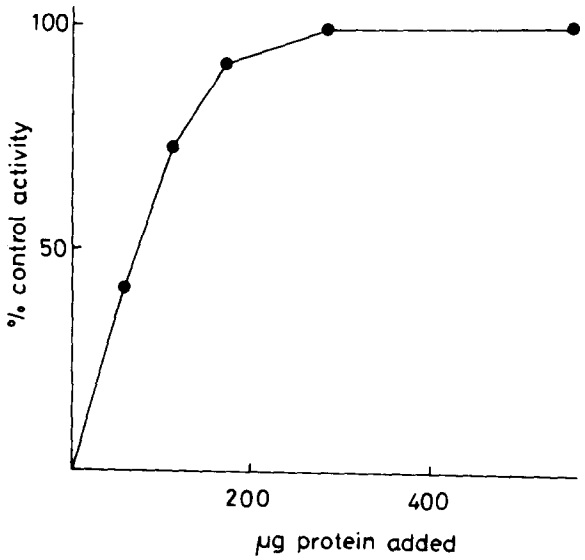

Fig.4. Reactivation of elastase-inactivated fatty acid synthetase by medium-chain thioesterase. Fatty acid synthetase was completely inactivated by digestion with elastase for 60 min, as described in fig.1 legend. The graph shows the effect of adding various amounts of partially purified medium-chain thioesterase, and the results are expressed as a percentage of the activity of untreated fatty acid synthetase.

4. Discussion

The pattern of cleavage obtained during digestion of fatty acid synthetase with elastase is consistent with a model in which a first rapid cleavage of the 250 000 dalton subunit to fragments of 220 000 and 35 000 daltons is followed by a slower cleavage of the 220 000 dalton species to 120 000 and 110 000 dalton fragments. Thus the 2 subunits are not only identical in size but are cleaved by elastase at 2 identical positions in the chain. This cleavage pattern strongly supports the view that the subunits are very similar or identical.

The idea that the 2 subunits are identical is also supported by the findings that there are close to two molecules of the prosthetic group 4-phosphopantetheine/500 000 dimer [3,9], and that complete inactivation of the thioesterase component requires the covalent reaction of 2 molecules of phenylmethane sulphonyl fluoride [10], or diisopropyl fluorophosphate [11], per dimer.

Qureshi et al. [12] have claimed that two different subunits can be separated from avian and mammalian fatty acid synthetases using affinity chromatography. However as they did not estimate the molecular weights of the separated species, and since fatty acid synthetase is highly susceptible to proteolysis [2], it remains a possibility that their 'subunits' were proteolytic fragments of the enzyme. In view of the accumulating evidence that the subunits of fatty acid synthetase are identical it seems important for them to exclude this possibility.

We have also shown that the thioesterase component of fatty acid synthetase is located in a 35 000 dalton component which must be at, or close to, one end of the chain. Since this fragment has amino-terminal lysine, and we could not detect a free amino-terminal on the native enzyme, we would tentatively suggest that the thioesterase component is located near the carboxy-terminal end of the chain. Smith et al. [13–15] and Bedord et al. [16] have shown that a fragment of 30 000–35 000 daltons containing the thioesterase activity is released on treatment of mammalian or avian fatty acid synthetase with trypsin. However trypsin gives much more extensive fragmentation of the enzyme than does elastase (S. L. and D. G. H., unpublished results) and Bedord et al. [16] required several purification steps to obtain a homogeneous thioesterase fragment. Libertini and Smith [17] recently reported the reactivation of trypsin-inactivated fatty acid synthetase with medium-chain thioesterase. However they did not obtain 100% reactivation, probably because trypsin cleaves at more than one site.

It is clear that there is a single site on fatty acid synthetase close to the thioesterase component which is rapidly cleaved by elastase, but why this single cleavage should cause inactivation of the enzyme is not clear. The thioesterase activity is actually increased on digestion with elastase (fig.1), although this may merely be due to increased accessibility to the model substrate palmityl-CoA. The finding that the digested enzyme can be completely reactivated by addition of medium-chain thioesterase shows that the ability of the other 6 activities and the acyl carrier to synthesize medium-chain fatty acids is likewise not affected by elastase. This finding also suggests that the thioesterase is not the rate-limiting activity in the overall reaction.

Acknowledgements

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