Amino acid sequence around the active serine in the acyl transferase domain of rabbit mammary fatty acid synthase

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Rabbit mammary fatty acid synthase was labelled in the acyl transferase domain(s) by the formation of the *O*-ester intermediates after incubation with [^{14}C]acetyl- or malonyl-CoA. Elastase peptides containing the labelled acyl groups were isolated using high performance liquid chromatography and sequenced by fast atom bombardment mass spectrometry. An identical peptide (acyl-Ser–Leu–Gly–Glu–Val–Ala) was obtained after labelling with acetyl- or malonyl-CoA. This confirms the hypothesis that, unlike *Escherichia coli* or yeast, a single transferase catalyses the transfer of both acetyl- and malonyl-groups in the mammalian complex. The sequence at this site is compared with that around the active serine in other acyl transferases and hydrolases.

Amino acid sequence *Active serine* *Acyl transferase* *Fatty acid synthase*

1. INTRODUCTION

The fatty acid synthase complexes in different organisms occur in strikingly different structural forms. In *Escherichia coli* [1] and in the chloroplasts of green plants [2] the individual activities are located on discrete proteins. However, in yeast, the activities are located on only two different multifunctional polypeptide chains [3,4] while in vertebrates they are present on a single multifunctional polypeptide [5–7].

The reaction mechanisms catalysed by these different complexes are superficially similar. Acetyl- and malonyl-groups are transferred from CoA to an enzyme-bound phosphopantetheine group via acyl-enzyme intermediates which have the characteristics of *O*-esters [1,8–10]. The same partial reactions are thought to be involved in the subsequent elongation of the nascent fatty acid [11]. However, it is becoming clear that the mechanisms are not identical in all organisms. In vertebrates, the completed fatty acid is hydrolysed from the enzyme by a thioesterase to release free fatty acids, whereas in yeast it is transferred directly to CoA by a long chain acyl transferase [1]. Also, in yeast and in *E. coli*, separate acetyl- and malonyl-transferases catalyse the initial loading reactions [1], while in mammals kinetic evidence suggests that a single transferase may catalyse both reactions [11]. We have recently demonstrated that the acyl-*O*-esters formed on incubation of the mammalian enzyme with acetyl- and malonyl-CoA are true intermediates in the acyl transferase reactions [12] and have provided preliminary evidence that identical acyl peptides can be derived from enzyme labelled at the *O*-ester sites with acetyl- or malonyl-CoA [7]. In this paper we report the isolation and sequencing of these peptides and confirm that they are identical.

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2. MATERIALS AND METHODS

Fatty acid synthase was purified from lactating rabbit mammary glands as in [13]. Acetyl-CoA, malonyl-CoA and elastase (lot 100F-8075) were from Sigma (Poole, Dorset); Sephadex was from

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Pharmacia (Uxbridge, Middlesex) and radiochemicals were from Amersham International Bucks). Acetonitrile (HPLC grade S) and trifluoroacetic acid (sequencer grade) were from Rathburn Chemicals (Walkerburn, Peebles).

2.1. *Labelling of fatty acid synthase*

Fatty acid synthase (20 mg) was labelled with [^{14}C]acetyl- or [^{14}C]malonyl-CoA (0.1 mM, 5 Ci/mol or 0.4 Ci/mol) as in [7], except that the enzyme used was the high-M_r core purified by gel filtration after release of the thioesterase and acyl carrier domains by limited elastase digestion [14]. Control experiments showed that prior removal of these domains did not affect either the stoichiometry of labelling or the peptides produced. After labelling, the protein was precipitated by adding 5 vol. of ice-cold 5% trichloroacetic acid and centrifuged (12000 × *g*; 5 min; 4°C). The pellet was washed with 10 ml of ice-cold water and recentrifuged as above. The washed pellet was dissolved in 0.7 ml of 90% formic acid, diluted with 10 ml of water and freeze-dried.

2.2. *Digestion of labelled fatty acid synthase*

The freeze-dried protein was resuspended in 5 ml of 1% (w/v) ammonium bicarbonate, digested for 6 h at 37°C with elastase (100:7, w/w) and freeze-dried.

2.3. *Purification of labelled peptides*

The digest was fractionated by gel filtration on a column (70 × 1.3 cm) of Sephadex G-50 Superfine equilibrated with 5% (v/v) formic acid. The peak of radioactivity was freeze-dried and subjected to reversed-phase high-performance liquid chromatography (HPLC) using an Ultrasphere ODS column (25 × 0.46 cm) on a Beckman-Altex HPLC system. Peptides were eluted in 0.1% (v/v) trifluoroacetic acid using a linear gradient from 10–25% (v/v) acetonitrile. Radioactive peptides were further fractionated using a Waters I-60 protein column eluted using water containing 0.% (v/v) trifluoroacetic acid.

2.4. *Analysis of peptides*

Amino acid analysis was carried out using an LKB Biochrom 4400 analyser. The peptides were sequenced by fast atom bombardment mass spectrometry [15] in the negative ion mode; 3 nmol of each peptide was dispersed in a glycerol matrix and bombarded with a beam of xenon atoms at 9 kV. The mass spectra were recorded on a Kratos MS-50 instrument. For the sequencing, peptides derived from fatty acid synthase labelled with CoA esters at 0.4 Ci/mol were used.

3. RESULTS

3.1. *Purification of peptides*

Elution profiles for the purification of [^{14}C]acetyl peptides are shown in fig.1. The

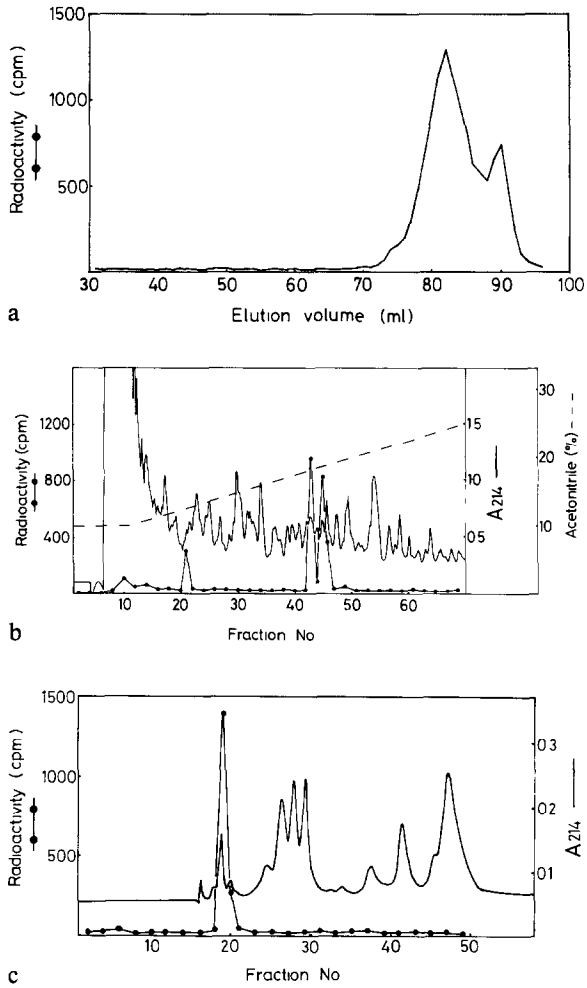


Fig.1. Elution of [^{14}C]acetyl peptides from: (a) Sephadex G-50; (b) Ultrasphere ODS; (c) I-60 columns. [^{14}C]Acetyl–CoA at 5 Ci/mol was used for enzyme labelling; (c) chromatography of Peak 2 from the Ultrasphere ODS eluate.

radioactivity eluted from the Sephadex G-50 column has one main peak with a shoulder. The shoulder disappeared on freeze-drying of the fractions and appears to be due to a small amount of breakdown of the acyl peptides to free acetate. Fig.1b shows the fractionation of the main peak by reversed-phase HPLC. Three main peaks of radioactivity were obtained. If a steeper gradient was used, the second and third peaks were not resolved, as shown in [7]. The 3 radioactive peaks were further purified by gel filtration on Waters I-60 columns. Results for peak 2 are shown in fig.1c but very similar results were obtained for all 3 peaks. Rechromatography on reversed-phase columns of any of the 3 peaks confirmed their purity after the I-60 step (not shown).

The results of purification of [^{14}C]malonyl peptide (not shown) were identical to those for the acetyl peptides except that consistently less of peak 1 was recovered.

3.2. *Analysis of peptides*

The compositions of the purified peptides are shown in table 1. The compositions were consistent with peaks 1, 2 and 3 being overlapping peptides of 2, 5 and 6 amino acids derived from a single site on the enzyme, and also with the acetyl and malonyl peptides being identical.

We were unable to find a free N-terminus on either of the penta- or hexapeptides using either the dansyl chloride reaction or automated liquid phase sequencing. These peptides were therefore sequenced using fast atom bombardment mass spectrometry. The data summarised in table 2 established the sequences of both the acetyl and malonyl hexapeptides to be *N*-acyl-Ser–Leu–Gly–Glu–Val–Ala. Results from experiments using the pentapeptides were consistent with this sequence. The N-terminal location of the acyl group was confirmed by showing that the molecular ions were identical after treatment with acetic anhydride, which would acetylate a free amino group.

Table 1

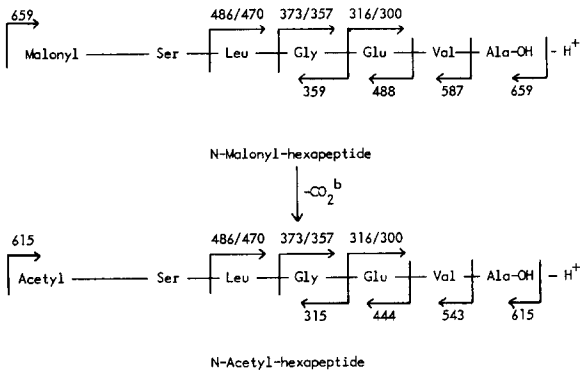
Amino acid compositions of peptides purified after elastase digestion of fatty acid synthase labelled with [^{14}C]acetyl-CoA or [^{14}C]malonyl-CoA

Amino acid	Acetyl peptides			Malonyl peptides	
	1	2	3	2	3
Ser	1.1	1.1	1.0	1.0	1.0
Glx	–	1.1	1.2	1.4	1.1
Gly	–	1.1	1.1	1.0	1.2
Ala	–	0.9	–	0.9	–
Val	–	0.9	1.0	1.0	0.9
Leu	1.0	1.0	1.0	1.0	1.0

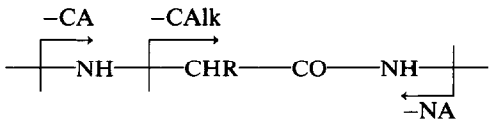
Values represent mol/mol of acyl group calculated from the radioactivity. Amino acids for which <0.1 mol/mol was determined are omitted. Hydrolysis was for 48 h (110°C, 6 M HCl). The yield of malonyl peptide 1 was too small to allow accurate amino acid analysis

Table 2

Amino acid sequence information[a] derived from negative ion FAB mass spectra of the malonyl- and acetyl-hexapeptides[b]



[a] Sequence ions observed in the negative ion FAB mass spectra of the above peptides are as follows [15]:



where the –CA and –NA cleavages occur with hydrogen transfer to the charged fragment

[b] Some decarboxylation of the malonyl group to produce an acetyl group was observed in the FAB mass spectra of the *N*-malonyl-hexapeptide

4. DISCUSSION

Purification of small peptides derived from a protein as large as fatty acid synthase is a difficult

Table 3

Comparison of amino acid sequences around the active site serine residue of some acyl transferases/hydrolases

Active site	Sequence	Ref.
Rabbit FAS (acyl transferase)	-SER-Leu-Gly-Glu-Val-Ala	[Here]
Yeast FAS (acetyl transferase)	Lys-SER-Gln-Gly-Leu-Val-Thr	[9]
Yeast FAS (malonyl transferase)	Ala-Gly-SER-Gly(His,Leu,Glx)Cys	[16]
Goose FAS (thioesterase)	-SER-Phe-Gly-Ala-Cys-Val	[18]
Bovine trypsin	Gly-Asp-SER-Gly-Gly-Pro-Val-Val	[19]

The serine at which the acyl-enzyme intermediate forms is shown in capitals. Identical residues are boxed. FAS = fatty acid synthase

task and was rendered feasible in this case by the remarkable purification afforded by the I-60 column run in 0.1% trifluoroacetic acid (fig.1c). Despite their small size, the di-, penta- and hexapeptides all eluted close to the void volume of the column. I-60 columns are believed to function as gel filtration columns when run at high ionic strength at neutral pH. The anomalous elution of these peptides under the present conditions may be due to the fact that their N-termini are blocked and they would contain no charged groups at pH 2. This may prove to be a general method for purification of blocked peptides containing no basic amino acids. Since the linkages between the acetyl and malonyl groups and intact fatty acid synthase are sensitive to hydroxylamine (1 M, pH 9.5, 2.0 h, 38°C; A.D.M., unpublished), the initial acylation cannot be at an amino group. An O ⟶ N migration must have occurred after proteolytic digestion, as reported for a peptide derived from the yeast acetyl transferase [9].

These results confirm that the sequences at the acetyl-*O*-ester and malonyl-*O*-ester sites on the mammalian enzyme are identical. Taken together with evidence that these *O*-esters are intermediates in the acyl transferase reactions [12], and that formation of acetyl-*O*-ester blocks formation of malonyl-*O*-ester and vice versa [11], this strongly suggests that in the mammalian complex a single acyl transferase catalyses transfer of both acetyl and malonyl groups to the acyl carrier. This contrasts with the situation in yeast and *E. coli* where separate transferases are involved [1].

Numerous other acyl transferases/hydrolases, including the serine proteases and the thioesterase of the vertebrate fatty acid synthase complex, have been shown to contain active serine residues which are believed to form acyl-enzyme intermediates. In table 3 the sequences around this active serine residue are compared for some acyl hydrolases and the acyl transferases of rabbit (this work) and yeast fatty acid synthases. Although the available sequences are too short to allow firm conclusions about evolutionary or mechanistic relationships, some interesting similarities are apparent. With the exception of the yeast malonyl transferase, all contain the sequence acyl-Ser–X–Gly. The mammalian acyl transferase sequence is remarkably similar to the yeast acetyl transferase sequence, and bears no resemblance to the yeast malonyl transferase sequence. It is interesting that in yeast the malonyl transferase also catalyses the terminating long chain acyl transferase step [16,17]. This latter activity is totally lacking in the vertebrate enzyme, which uses instead a thioester hydrolase reaction for chain termination.

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