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A novel acyl-CoA-binding protein from bovine liver

Effect on fatty acid synthesis

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Bovine liver was shown to contain a hitherto undescribed medium-chain acyl-CoA-binding protein. The protein co-purifies with fatty-acid-binding proteins, but was, unlike these proteins, unable to bind fatty acids. The protein induced synthesis of medium-chain acyl-CoA esters on incubation with goat mammary-gland fatty acid synthetase. The possible function of the protein is discussed.

INTRODUCTION

Termination of fatty acid synthesis at medium chain length by goat mammary-gland fatty acid synthetase is caused by a medium-chain acyltransferase, and the resulting product is acyl-CoA esters (Knudsen & Grunnet, 1982). The acyltransferase involved is an inherent part of the synthetase complex and is identical with the loading transferase (Mikkelsen *et al.*, 1985*a*,*b*). In order to function in termination, the transferase requires the presence of an acyl-CoA-binding or -removing system such as bovine serum albumin, methylated α-cyclodextrins or the microsomal triacylglycerol-synthesizing system (Knudsen & Grunnet, 1982; Hansen *et al.*, 1984*a*,*b*).

It has been suggested that fatty-acid-binding proteins (FABPs) influence the incorporation of long-chain fatty acids into triacylglycerols in liver and intestine (O'Doherty & Kuksis, 1975; Burnett *et al.*, 1979; Mishkin & Turcotte, 1974). These FABPs may act as specific transfer proteins for fatty acids or acyl-CoAs (Bass, 1985). Two FABPs, of pI 6.0 and pI 7.0, have been purified and characterized (Haunerland *et al.*, 1984; Keuper *et al.*, 1985).

In the present experiment we have tested the ability of FABP to bind medium-chain acyl-CoAs and thereby induce medium-chain fatty acid synthesis by goat mammary-gland fatty acid synthetase.

MATERIALS AND METHODS

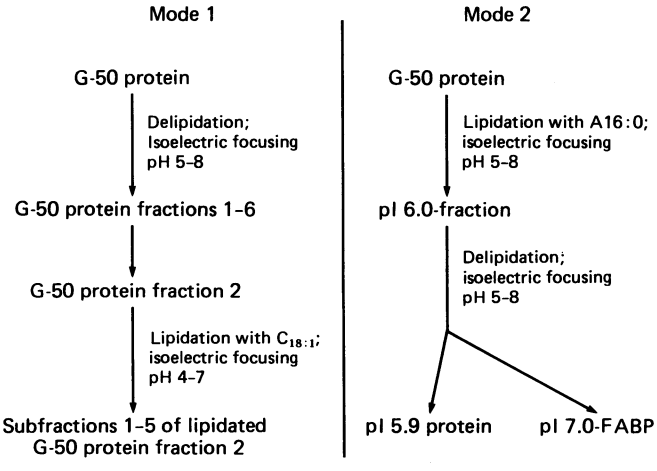
Goats of mixed breed 4 weeks *post partum* were used. NADPH, dithiothreitol, α-cyclodextrin and M_r standards for polyacrylamide-gel electrophoresis were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. [1-^{14}C]Acetyl-CoA was synthesized enzymically as described by Hansen *et al.* (1984*a*). Malonyl-CoA was synthesized by the method of Eggerer & 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). Chemicals for purification of FABP were obtained as described by Haunerland *et al.* (1984). The pI calibration kit was purchased from Pharmacia, Freiburg, West Germany. Goat mammary gland was homogenized and subcellularly fractionated, and fatty acid synthetase purified as described by Knudsen (1972). The pI 7.0-FABP was purified as described by Haunerland *et al.* (1984); for identification of the inducing factor, the Bio-Gel P10 column in the published method was replaced by a Sephadex G-50 column. Subfractions of the freeze-dried Sephadex G-50 eluate containing the FABPs were obtained by two different modes of isoelectric focusing as outlined in Scheme 1, both carried out as described by Haunerland *et al.* (1984).

Incubation with fatty acid synthetase

Goat mammary-gland fatty acid synthetase was incubated and products were analysed as described previously (Knudsen & Grunnet, 1982).

Determination of fatty acid binding to proteins

[1-^{14}C]Oleic acid (0.75 nmol) in hexane was placed into a 2 ml glass tube and the solvent was removed in a stream of N_2. Protein fractions (20–50 μg) dissolved in water



Scheme 1. Flow scheme for subfractionation of Sephadex G-50 protein fraction

Abbreviation used: FABP, fatty-acid-binding protein.

Table 1. Effect of G-50 protein on fatty acid synthesis by lactating goat mammary-gland fatty acid synthetase

Fatty acid synthetase (155 μg; sp. activity 1130 nmol of NADPH oxidized/min per mg) was incubated as described in the Materials and methods section. Sephadex G-50 protein and 2,6-di-*O*-methyl-α-cyclodextrin were added as indicated. Values for [1-^{14}C]acetate incorporation are means of duplicates ± half the difference. Values for radioactivity distribution are means of duplicates. The results in the Table are typical for four different G-50 protein preparations.

Additions		Total acetate incorporated from [1-^{14}C]acetyl-CoA (nmol)	Distribution of radioactivity (%)						
G-50 protein (mg/ml)	2,6-Di-*O*-methyl-α-cyclodextrin (mg/ml)		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}
—	—	1.4±0.06	30	6	4	4	13	39	4
—	5.0	2.3±0.09	32	13	15	29	5	6	—
3.0	—	1.8±0.04	33	10	7	22	12	13	3

Table 2. Effect of G-50 protein subfractions from isoelectric focusing on the pattern of fatty acids synthesized by goat mammary-gland fatty acid synthetase

Fatty acid synthetase (185 μg; sp. activity 1220 nmol of NADPH oxidized/min per mg) was incubated as described in the Materials and methods section. G-50 subfractions were added as indicated. Values for [1-^{14}C]acetate incorporation are means of duplicates ± half the difference. Values for radioactivity distribution are means of duplicates. The results are typical for subfractions of two different G-50 protein preparations.

Additions (mg/ml)	Fatty acid binding	Total acetate incorporated from [1-^{14}C]acetyl-CoA (nmol)	Distribution of radioactivity (%)						
			C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}
—	−	1.40±0.06	26	9	5	10	23	23	4
G-50 protein (0.8)	++	1.36±0.01	24	10	8	22	22	13	1
G-50 protein fractions									
1. pH 4.2–5.7 (0.4)	+	1.16±0.05	27	11	7	11	23	19	1
2. pH 5.7–6.6 (0.4)	++	1.82±0.10	35	13	12	25	7	6	2
3. pH 6.6–6.9 (0.4)	−	1.18±0.14	34	12	12	22	14	3	2
4. pH 6.9–7.2 (0.4)	++	1.45±0.04	35	11	8	9	19	15	4
5. pH 7.2–7.7 (0.4)	−	1.44±0.01	36	9	9	11	21	13	1
6. pH 7.7–8.2 (0.4)	−	1.26±0.02	36	12	5	10	20	15	1

Table 3. Effect of subfractions of lipidated G-50 protein fraction 2 from Table 2 (pH 5.7–6.6) on the fatty acid synthesis by fatty acid synthetase from goat mammary gland

Fatty acid synthetase (64 μg; sp. activity 1150 nmol of NADPH oxidized/min per mg) was incubated as described in the Materials and methods section. Proteins were added as indicated. Values for total acetate incorporation are means of duplicates ± half the difference. Values for radioactivity distribution are means of duplicates. The results are typical for incubations with subfractions from two different pH 5.7–6.6 fractions.

Additions (mg/ml)	Fatty acid binding	Total acetate incorporated from [1-^{14}C]acetyl-CoA	Distribution of radioactivity (%)						
			C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}
—	−	1.40±0.06	26	9	5	10	23	23	4
G-50 fraction 2 (pH 5.7–6.6)	++	1.82±0.1	35	13	12	25	7	6	2
Subfractions									
1. pH 4.8–4.9 (0.24)	−	1.21±0.10	39	12	5	10	16	11	7
2. pH 4.9–5.2 (0.32)	+	1.16±0.06	38	13	7	10	18	5	—
3. pH 5.2–5.5 (0.34)	++	1.36±0.04	36	12	9	10	20	11	1
4. pH 5.5–5.7 (0.28)	+	1.38±0.05	34	11	10	24	15	5	—
5. pH 5.7–6.6 (0.39)	−	1.30±0.10	32	14	12	25	11	5	1
pI 5.9 protein (0.40)	−	1.73±0.08	28	15	14	28	10	4	1
pI 7.0-FABP (0.40)	++	2.29±0.12	31	10	8	11	22	15	3

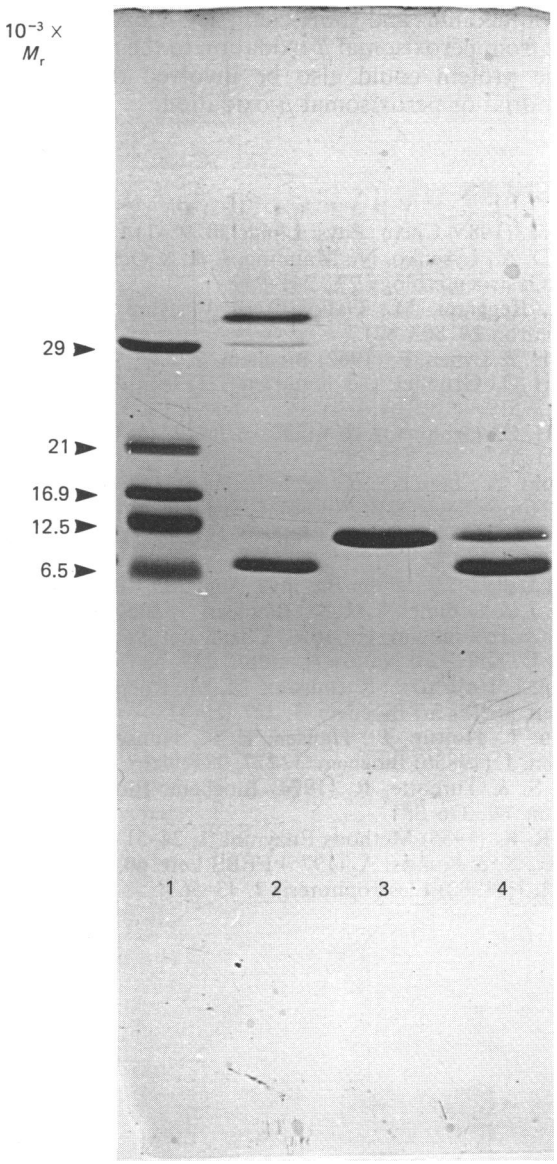


Fig. 1. SDS/polyacrylamide-gel electrophoresis of G-50 protein subfractions

This was performed as described in the Materials and methods section. Lane 1, M_r markers. Lane 2, subfraction 5 from Table 3. Lane 3, pI 7.0-FABP. Lane 4, the pI 5.9 protein from Table 3.

were added and incubated for 15 min at room temperature; samples (15–30 μl) were then subjected to isoelectric focusing on ultrathin polyacrylamide gels (100 μm), which were prepared and focused as described by Radola (1980). pH gradients were determined with the aid of the IEF calibration kit pH 3–10. Proteins were stained as described by Radola (1980), after the dried gels were subjected to autoradiography. Binding of ligand to FABP was indicated by superposition of protein stain and the autoradiographic track of the ligand. Weak staining is indicated by + and heavy staining by ++, by visual inspection of the film.

Polyacrylamide-gel electrophoresis

This was carried out in the presence of SDS essentially as described by Laemmli (1970). The gel used was 20% acrylamide with 0.8% bisacrylamide. The M_r markers were carbonic anhydrase (29000), soya-bean trypsin inhibitor (21000), myoglobin (16900), cytochrome *c* (12500) and aprotinin (6500).

RESULTS AND DISCUSSION

Incubation of goat mammary-gland fatty acid synthetase with G-50 protein containing both pI 6.0-FABP and pI 7.0-FABP resulted in increased medium-chain fatty acid synthesis (Table 1). The G-50 protein (3 mg/ml) was as effective as 2,6-di-*O*-methyl-α-cyclodextrin (5 mg/ml) in shifting the composition of fatty acids synthesized from long-chain to medium-chain fatty acids.

In the first attempt to identify the protein involved, the G-50 protein was delipidated (Morton, 1955) and subjected to isoelectric focusing in a pH 5–8 gradient. The resulting G-50 protein fractions 1–6 were analysed for their ability to bind fatty acids and incubated with goat mammary fatty acid synthetase (Table 2). Protein focusing between pH 5.7 and 6.9 (fractions 2 and 3) was able to induce medium-chain fatty acid synthesis by the goat mammary synthetase. Fraction 2 was also able to bind fatty acids, because it contains the pI 6.0-FABP. Lipidation of the pI 6.0-FABP with oleic acid changes its pI from 6.0 to 5.0–5.1 (Haunerland *et al.*, 1984). The inducing G-50 protein fraction 2 was therefore lipidated with oleic acid and refocused in a pH 4–7 gradient. After termination of the run, the pH in the gel was measured and the subfractions 1–5 shown in Table 3 were scraped off. Proteins were eluted from the gel with 2% Servalyte pH 4–7, as this ampholyte stabilizes pI 6.0-FABP (Haunerland *et al.*, 1984). The subfractions from this second isoelectric focusing were also incubated with goat mammary synthetase (Table 3). The results show that the inducer protein's pI was unaffected by lipidation; it still appeared in the fractions focusing between pH 5.5 and 6.6. The results therefore show that the pI 6.0-FABP cannot be the inducer protein, because this protein is present mainly in subfractions 2 and 3 (pH 4.9–5.5). That this also is the case is indicated by the ability of these fractions to bind fatty acids. In contrast, the pH 5.5–6.6 fraction, which strongly induces medium-chain fatty acid synthesis, does not bind fatty acid in detectable amounts. All the protein subfractions from the second run contained 2% pH 4–7 Servalyte, as mentioned, and it was shown that this Servalyte concentration did not affect the pattern of fatty acids synthesized (results not shown).

Further evidence that it is neither the pI 6.0-FABP nor the pI 7.0-FABP that acts as medium-chain acyl-CoA binder, and thereby as inducer of medium-chain fatty acid synthesis, comes from incubations of goat mammary-gland fatty acid synthetase with the pI-5.9 protein and the pI 7.0-FABP resulting from the second mode of isoelectric focusing of the G-50 protein. The G-50 protein was here initially focused after lipidation with 16-(9′-anthroyloxy)palmitic acid (A16:0). When lipidated with this compound, the pI 6.0-FABP focuses at pH 5.0–5.1 and the pI 7.0-FABP at 6.0–6.1 (Haunerland *et al.*, 1984). The pI 6.0 fraction (pH 5.8–6.3) scraped off in the first run will therefore contain both the unknown inducer protein and the pI 7.0-FABP, but not the pI 6.0-FABP, which focuses around pH 5.0 under these conditions. After delipidation and refocusing in a pH 5–8 gradient, the pI 7.0-FABP focuses at around pH 7.0 and

the unknown inducer protein should stay around pH 5.9. The results in Table 3 show that the pI 5.9 protein can induce and that the pI 7.0-FABP is unable to do so. That the pI 6.0-FABP is not present in the pI-5.9 protein fraction can be seen from the inability of this fraction to bind fatty acids.

Polyacrylamide-gel electrophoresis showed that both the pI 5.9 protein and the subfraction 5 contain a protein which migrates faster than the pI 7.0-FABP (Fig. 1). The slightly faster-migrating protein represents the inducer protein, because it is the only major protein that is common to both protein fractions. The M_r calculated from migration of the M_r markers is 7000 ± 500.

The product of medium-chain fatty acid synthesis by goat mammary-gland fatty acid synthetase is, as mentioned in the introduction, medium-chain acyl-CoA esters. Analysis by h.p.l.c. of the products synthesized in the presence of the medium-chain inducing factor showed that the medium-chain fatty acids were released as acyl-CoA esters. Only trace amounts of non-esterified fatty acids were found in the incubation medium (results not shown).

We can therefore conclude that the G-50 protein fraction contains, besides the pI 6.0- and pI 7.0-FABP, a novel, hitherto undescribed, acyl-CoA-binding protein.

The possible function of this protein in bovine liver is unknown. It is unlikely that the protein functions as a carrier of medium-chain acyl-CoA esters from fatty acid synthetase to the microsomal triacylglycerol-synthesizing system, because the liver does not synthesize medium-chain fatty acids.

It might be speculated that the protein is involved in transfer of medium- and short-chain acyl-CoA ester end products from peroxisomal β-oxidation to the mitochondria. The protein could also be involved directly in mitochondrial or peroxisomal β-oxidation.

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