Biochem. J. (1989) 262, 513–519 (Printed in Great Britain)

Acyl-CoA-binding protein in the rat

Purification, binding characteristics, tissue concentrations and amino acid sequence

Jens KNUDSEN,*§ Peter HØJRUP,† Hans Otto HANSEN,* Hans Frede HANSEN‡ and Peter ROEPSTORFF†
*Institute of Biochemistry and †Institute of Molecular Biology, Odense University, DK-5230 Odense M, Denmark, and ‡Department of Clinical Chemistry, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark

Acyl-CoA-binding protein (ACBP) was purified from rat liver. The M_r was determined as 9932 ± 10 by mass spectrometry and calculated as 9937.8 from the sequence. The protein binds acyl-CoA esters (C_8–C_{16}) with high affinity, but was unable to bind fatty acids. ACBP was found mainly (86%) in the soluble fraction, and the concentration was highest in liver, 5–6 μg/mg of soluble protein. The complete primary structure was determined by a combination of gas-phase Edman degradations and mass spectrometry. Extensive use of ^{252}Cf plasma-desorption mass spectrometry facilitated the identification and verification of peptides. Comparison with the previously determined sequence of bovine acyl-CoA-binding protein revealed a very strong sequence similarity (83%), and all of the differences could be accounted for by single base changes.

INTRODUCTION

We previously identified and purified a novel low-M_r acyl-CoA-binding protein (ACBP) from bovine liver (Mogensen *et al.*, 1987). The protein, which has an average M_r of 9955, binds acyl-CoA with a high affinity, but is unable to bind fatty acids (Mikkelsen *et al.*, 1987; Mikkelsen & Knudsen, 1987). The biological assay for the protein is based on its ability to induce medium-chain fatty acid synthesis by goat mammary-gland fatty acid synthetase (Mogensen *et al.*, 1987). The amino acid composition and primary structure of the protein have been determined (Mikkelsen *et al.*, 1987). The amino acid sequence showed no homology with published protein sequences. ACBP was found in all tissues tested, but the highest concentration was found in liver (3.3 μg/mg of soluble protein) (Mikkelsen & Knudsen, 1987). No other information has appeared on ACBP in the biochemical literature.

Further information on the physiological function of ACBP may be obtained through determination of the effect of nutritional status and hormonal treatment on tissue concentrations of ACBP and ACBP mRNA. However, ruminants are unsuitable for this purpose. We therefore changed the experimental animal from the cow to the rat. In the present paper we report purification of rat liver ACBP, its full primary amino acid sequence, binding characteristics and tissue distribution and concentration. The results show that rat liver ACBP amino acid sequence is 83% identical with the sequence of bovine liver ACBP. The binding characteristics, tissue concentration and distribution are similar to those found for bovine liver ACBP.

MATERIALS AND METHODS

Enzymes and special reagents

Chymotrypsin (EC 3.4.21.1) and trypsin (EC 3.4.21.4) were from E. Merck, and *Staphylococcus aureus* V8 proteinase (SAP) (EC 3.4.21.19) was from Miles Laboratories. Sephadex G-50 and G-100 were from Pharmacia Biotechnology International AB, Uppsala, Sweden, Nucleosil ODS (10 μm particle size; 10 and 30 nm pore size) packing was from Macherey–Nagel, Düren, Germany. Propan-2-ol (h.p.l.c. grade) and trifluoroacetic acid were from Rathburn, Walkerburn, Scotland, U.K. All other chemicals used in this study were of analytical grade.

Preparation of G-50 protein

A G-50 fraction, which contained both the fatty acid-binding protein and ACBP, was obtained from rat liver as described for bovine liver by Haunerland *et al.* (1984), except that a Sephadex G-100 column and a Sephadex G-50 (superfine-grade) column were used instead of a Sephacryl S-200 column and a Bio-Gel P-10 column respectively.

Purification of ACBP

The freeze-dried G-50 fraction was dissolved in buffer A [0.1% (v/v) trifluoroacetic acid in water] and centrifuged at 18 000 *g* for 2 min before injection on an h.p.l.c. column (120 mm × 8 mm) packed with Nucleosil ODS. The column was equilibrated in 20% (v/v) buffer B [0.1% trifluoroacetic acid in 50% (v/v) propan-2-ol] in buffer A with a flow rate of 3.0 ml/min. The proteins were eluted with a gradient of buffer B as shown in Fig. 1.

Digestion with *Staphylococcus aureus* proteinase

A 0.6 mg portion of rat ACBP was dissolved in 0.5 ml of 0.05 M-ammonium acetate buffer, pH 4.0; 30 μg of SAP was added, and the digestion was allowed to proceed for 4 h before another 30 μg of enzyme was added. The digestion was allowed to proceed for another 16 h at

Abbreviations used: ACBP, acyl-CoA-binding protein; DBI, diazepam-binding inhibitor; SAP, *Staphylococcus aureus* protease.
§ To whom reprint requests should be addressed.

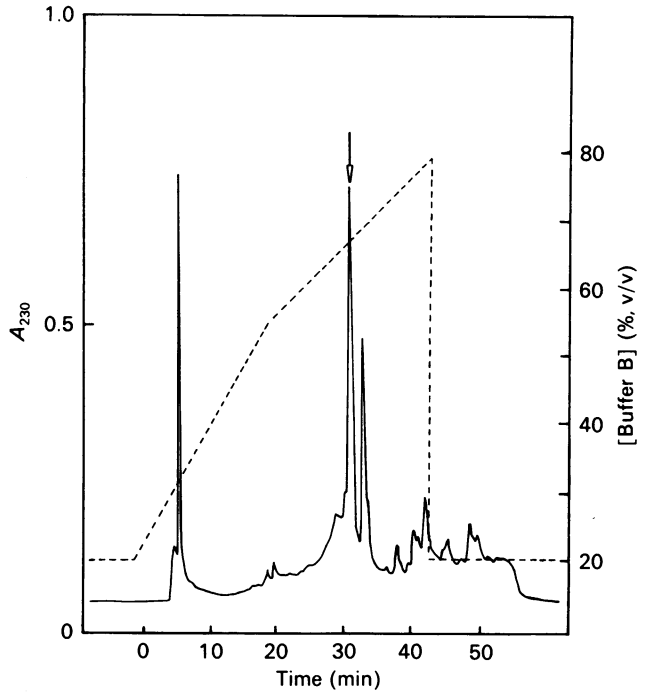

Fig. 1. Purification of ACBP by reverse-phase h.p.l.c.

The column (120 mm × 8 mm) was packed with Nucleosil ODS. The proteins were eluted with a gradient of buffer B in buffer A (----). The ACBP-containing peak is marked with an arrow. For experimental details see the Materials and methods section.

37 °C until terminated by injection of the sample on h.p.l.c.

Digestion with trypsin and chymotrypsin

A 0.5 mg portion of rat ACBP was dissolved in 0.5 ml of 0.05 M-NH_4HCO_3/0.1 mM-$CaCl_2$, pH 8.2; 20 μg of trypsin was added, and the reaction was allowed to proceed for 90 min at 37 °C until terminated by injection on h.p.l.c. Sub-digestion of SAP peptides was carried out in 100 μl of the same buffer with 2 μg of trypsin or chymotrypsin for 15–60 min at room temperature.

Purification of peptides

Peptides were separated on a 4 mm × 250 mm Knauer column packed with Nucleosil 300 C_{18}, 10 μm particle size. The column was equilibrated with 95% buffer A (0.1% trifluoroacetic acid) and 5% buffer B (0.1% trifluoroacetic acid in propan-2-ol) and eluted with a gradient of buffer B in buffer A (5–15% buffer B for 10 min, followed by 15–35% buffer B for 10 min).

Amino acid analysis

Peptide and protein samples (0.1–2 nmol) were hydrolysed in the gas phase of 6 M-HCl/0.1% phenol at 110 °C for 18–24 h in closed containers under reduced pressure. After freeze-drying, the samples were redissolved in 60 mM-sodium citrate buffer, pH 2.20, and portions (two-thirds to one-twentieth) were loaded on to an LKB Alpha amino acid analyser equipped with post-column *o*-phthaldehyde derivative formation and fluorescent detection. The result for the whole protein represents the average of four separate analyses, whereas those for the peptides represent single-sample analyses. No corrections were made for hydrolytic loss or incomplete hydrolysis.

Determination of amino acid sequences

Peptides were sequenced on an Applied Biosystems gas-phase Sequenator with chemicals and programme (MHTFA1) as recommended by the manufacturer. A 0.1–2 nmol portion of peptide was used for each sequence run, and the amino acid phenylthiohydantoin derivatives were identified on an HP 1084B instrument with a Beckman ODS column (4 mm × 250 mm) and a gradient of acetonitrile in 35 mM-sodium acetate buffer, pH 5.2, containing 1% (v/v) tetrahydrofuran (Hunkapiller & Hood, 1983).

Mass spectrometry (m.s.)

The M_r of the intact rat ACBP and of all peptides was determined on a BioIon BIN 10K plasma-desorption time-of-light mass spectrometer. The samples were dissolved in 0.1% trifluoroacetic acid and 10–100 pmol was applied in 2–3 μl on to an aluminized Mylar foil coated with nitrocellulose and spin-dried (Nielsen *et al.*, 1988). After insertion of the sample in the mass spectrometer, it was bombarded with fission fragments from a 10 μCi ^{252}Cf source. The spectra of peptides were recorded for 5×10^5 primary ions, whereas the spectra of the intact protein were recorded for 2×10^6 primary ions. M_r for the intact protein is average M_r calculated from the single-, double- and triple-charged molecular ions.

Electron-impact m.s. of the blocked *N*-terminal tryptic peptide was performed on a Finnigan MAT 311A mass spectrometer coupled to an SS200 data-acquisition system. The mass spectrometer was operated at a resolution of 1000 and 70 eV electron energy. The sample (approx. 10–20 nmol) was *N*-acetylated and *NO*-permethylated by the procedure previously described (Roepstorff, 1978).

Polyacrylamide-gel electrophoresis

This was carried out essentially as described by Laemmli (1970). The M_r markers used were myosin (M_r 200000), α_2-macroglobulin (M_r 180000), phosphorylase *a* (M_r 94000), bovine serum albumin (M_r 66000), ovalbumin (M_r 43000), carbonic anhydrase (M_r 29000), cytochrome *c* (M_r 11700) amd aprotinin (M_r 6500).

Fluorescence measurements

These were carried out on a Spex Fluorolog 2 spectrofluorimeter at 20 °C. To the cuvette, containing 1.2 ml of 20 mM-potassium phosphate buffer, pH 7.0, and 10 μM-ACBP, were added increasing amounts of different acyl-CoAs or non-esterified fatty acids in small volumes (5–40 μl) of 20 mM-potassium phosphate buffer, pH 7.0. The total volume of added acyl-CoA solution did not exceed 0.14 ml, and the fluorescence values were corrected for the dilution effect.

Production and detection of antibody

Rabbits were injected once with 200 μg of ACBP dissolved in 0.5 ml of distilled water and mixed with equal volumes of Freund's complete adjuvant, and five times with 200 μg of ACBP mixed with equal volumes of Freund's incomplete adjuvant.

Antibody titre against ACBP was determined by e.l.i.s.a (Tanaka *et al.*, 1985). Micro-titre plates were coated with ACBP (0.4 μg) in 100 μl of 50 mM-Na_2CO_3

buffer (pH 9.5)/well, by incubating overnight. After unbound ACBP was washed away with PBS (10 mM-potassium phosphate/137 mM-NaCl/2.5 mM-KCl, pH 7.5), plates were incubated with a blocking solution (1% bovine serum albumin in PBS) for 3 h at 37 °C to prevent non-specific binding of antiserum. Antiserum diluted in 0.1% bovine serum albumin in PBS was added and the samples were incubated overnight. Antibody binding was detected by using alkaline-phosphatase-conjugated affinity-purified pig anti-rabbit IgG. Binding was detected by addition of 200 μl of substrate solution [1.0 mg of *p*-nitrophenyl phosphate in 0.1 M-ethanolamine (pH 9.8)/0.5 μM-$MgCl_2$]. Preimmune serum from the rabbit served as a negative control and gave readings that were less than 1% of those with immune serum at the same dilutions.

Affinity purification of anti-ACBP antibodies

ACBP (4.0 mg) was coupled to 1 g of CNBr-activated Sepharose 4B essentially as recommended by the manufacturer (Pharmacia). The ACBP-specific antibody was purified on this column.

Preparation of subcellular fractions of rat liver

Sprague–Dawley rats (200 g) fed on normal rat chow were anaesthetized with pentobarbitol (0.7 ml). The liver was removed and perfused with 20 mM-potassium phosphate (pH 7.4)/150 mM-NaCl to remove blood. Afterwards it was homogenized in 2 vol. of the same buffer in a Potter homogenizer (0.5 mm clearance). Subcellular fractions were obtained by differential centrifugation as follows: nuclei 600 *g* for 10 min, mitochondria 4000 *g* for 10 min, lysosome–peroxisome fraction 25000 *g* for 10 min and microsomal fraction 100000 *g* for 10 min. All fractions were suspended in the above buffer and stored at −80 °C.

Preparation of 10000 *g* supernatant from heart, kidney, muscle and intestine

The tissues were cut into small pieces with a pair of scissors, placed in 2 vol. of 20 mM-potassium phosphate (pH 7.4)/150 mM-NaCl, and homogenized with an Ultra-Turrax homogenizer at full speed for 2 min. The homogenate was centrifuged at 10000 *g* for 30 min. The supernatant was stored at −80 °C.

Preparation of erythrocyte lysates

Sprague–Dawley rats (200 g) were anaesthetized as above, and blood was withdrawn from the heart with a syringe into heparin-containing test tubes and centrifuged at 1500 *g* for 10 min. Plasma and buffy coat were removed. The erythrocytes were washed in 9 vol. of 20 mM-potassium phosphate (pH 7.4)/150 mM-NaCl buffer. The washed erythrocytes were lysed in 9 vol. of 20 mM-KCl/20 mM-sucrose/10 mM-Tris/HCl, pH 7.4, for 15 min. Membranes were removed by centrifugation at 2000 *g* for 20 min, and the supernatant was stored at −80 °C until e.l.i.s.a could be performed.

E.l.i.s.a. of ACBP

ACBP was assayed by a multiple-layer enzyme-linked immunochemical procedure as described by Pawlak & Smith (1986), except that reaction of ACBP with immobilized rabbit anti-ACBP was carried out in 10 mM-Tris/HCl (pH 9.0)/0.5 M-NaCl/2.6 mM-KCl/0.1% bovine serum albumin/0.02% NaN_3 overnight at 4 °C. The layers, attached sequentially to a 96-well plastic plate, consisted successively of monospecific rabbit anti-ACBP IgG, ACBP antigen, biotinylated rabbit anti-ACBP IgG, avidin and biotinylated alkaline phosphatase; the chromogenic substrate was *p*-nitrophenyl phosphate.

Protein determination

The protein content in the ACBP solutions used was calculated from the amino acid content (see 'Amino acid analysis' above).

RESULTS AND DISCUSSION

Amino acid sequence

The complete sequence of rat liver ACBP is given in Fig. 2. The protein contains 86 residues, has an acetylated *N*-terminal serine residue and an average M_r of 9938.3 as calculated from the sequence. This value is in good agreement with that obtained by plasma-desorption m.s. of the intact molecule (9932 ± 10). The amino acid composition as calculated from acid hydrolysis (Table 1) fits within 10% with that calculated from the determined sequence, except that the values for serine and methionine are too low, as no correction for hydrolytic loss was made.

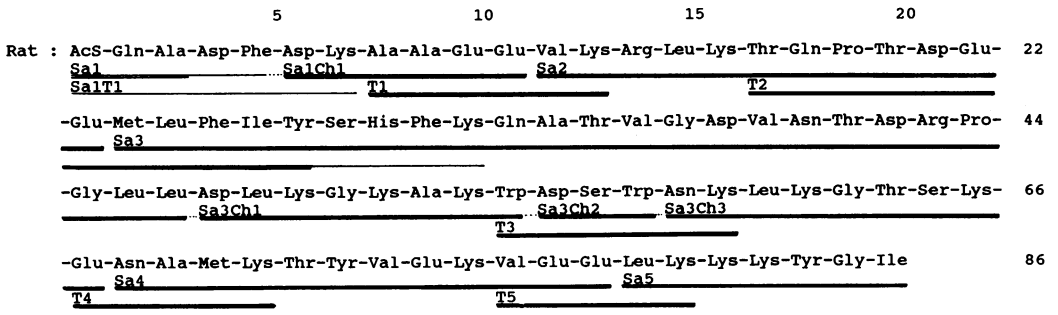

Fig. 2. Primary structure of rat liver ACBP and the peptides used to construct the amino acid sequence

Peptides are numbered from the *N*-terminus. SAP peptides have the prefix Sa, and the tryptic peptides the prefix T. SAP peptides sub-digested with chymotrypsin and trypsin have the prefixes SaCh and SaT respectively. Peptides were sequenced either by electron-impact m.s. (double line) or Edman degradation (bold line). The M_r of all peptides was checked by plasma-desorption m.s. Abbreviation: AcS, *N*-terminally acetylated serine.

Table 1. Amino acid composition and M_r of rat and bovine liver ACBP

N.D., not determined. The M_r values represent average values calculated from the sequence.

Amino acid	Amino acid composition (residues/molecule) calculated from		
	Amino acid analysis	Rat ACBP sequence	Bovine ACBP sequence
Asx	9.5	10	9
Thr	5.7	6	4
Ser	2.8	4	3
Glx	11.6	11	12
Gly	4.6	5	5
Ala	5.6	6	9
Cys	0.0	0	0
Pro	N.D.	2	2
Val	5.1	5	3
Met	1.5	2	3
Ile	2.3	2	4
Leu	7.5	7	5
Tyr	2.9	3	4
Phe	3.2	3	3
Lys	13.7	15	15
His	1.1	1	2
Arg	2.2	2	1
Trp	N.D.	2	2
M_r...		9938.3	9955.3

Digestion of rat liver ACBP with SAP at pH 4.0 cleaved the protein into five peptides, as cleavage occurred after Glu_{67} and the three occurrences of -Glu-Glu-. No cleavage was observed after Glu_{75}, probably because of the following basic lysine residue. The peptides were isolated by h.p.l.c. (Fig. 3), and no minor cleavage products were found. By adding the masses of the five peptides isolated (Table 2) and subtracting the mass of 4 water molecules (introduced by the hydrolytic cleavages), an M_r of 9937.8 for the whole digest is obtained. This value is so close to that determined for the intact molecule that it shows the five peptides to constitute the complete sequence.

As the *N*-terminus of rat liver ACBP is blocked (like its bovine counterpart; Mikkelsen *et al.*, 1987), no Edman degradation could be carried out on the intact protein or on peptides containing the *N*-terminal residue. The *N*-terminal SAP peptide, containing residues 1–11, was too large for electron-impact m.s., so the peptide was subdigested with chymotrpysin. This resulted in cleavage after the phenylalanine residue in position 5. However, the yield of the shortened peptide was too low for electron-impact m.s., but residues 6–11 could be sequenced with a gas-phase sequencer. An alternative tryptic subdigest was carried out with good yield of the residues 1–7 peptide.

A 30 nmol portion of this peptide was *N*-[$^2H_3$]acetylated and *NO*-permethylated. In the spectrum obtained by electron-impact m.s., peaks were obesrved at *m/z* 148, 328 and 413, showing the presence of Ac-Ser-Glu-Ala without the acetyl group being introduced during derivative formation. The presence of aspartic acid and phenylalanine in positions 4 and 5 respectively has not been confirmed by sequencing, but their identification

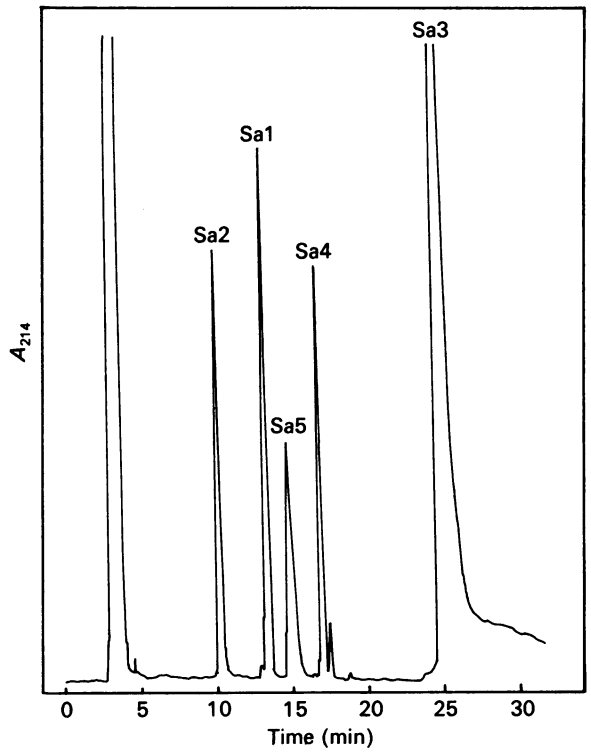

Fig. 3. Reverse-phase h.p.l.c. separation of peptides resulting from degradation of rat liver ACBP with SAP

Separation was carried out on a column (4 mm × 250 mm) packed with Nucleosil ODS (5 μm particle size; 30 nm pore size) with a linear gradient of propan-2-ol in 0.1% trifluoroacetic acid. For experimental details see the Materials and methods section.

can be identified as follows. The presence of Asp and Phe is established by amino acid analysis and m.s. (Table 2). Methylation analysis by plasma-desorption m.s. showed that the Asx determined by amino acid analysis is Asp and not Asn (results not shown). The positioning of Phe is shown by the ability of chymotrypsin to cleave after position 5.

The remainder of the sequence was obtained by Edman degradation of the SAP peptides. The large peptide Sa3 was sub-digested with chymotrypsin in order to obtain the *C*-terminal 17 residues of this peptide. Alignment of the SAP peptides was carried out by comparison with the bovine ACBP sequence, and the final sequence was checked by plasma-desorption by m.s./Edman degradation of peptides obtained from a tryptic digest of the intact protein.

Comparison of rat liver ACBP and bovine liver ACBP shows a very high degree of similarity (Fig. 4): 71 residues out of 86 residues are identical (82.6%), and all the substitutions can be explained by single-base pair changes. A computer search of the Protein Identification Resource (May, 1987) did not reveal any sequences homologous to rat liver ACBP.

Binding characteristics

The ability of different acyl-CoA esters to quench the intrinsic fluorescence at 332 nm from the ACBP was used as a quantitative indicator of the relative affinity of the protein for acyl-CoA esters with different chain length. All tested acyl-CoA esters with a chain length greater

Table 2. Amino acid composition and M_r values of SAP peptides isolated from rat ACBP

Values in parentheses correspond to the number of residues obtained by sequencing. N.D., not determined. All M_r values are average.

Amino acid	Peptide no....	Amino acid composition (residues/molecule)				
		1	2	3	4	5
Asx		1.92 (2)	1.0 (1)	5.87 (6)	0.85 (1)	
Thr			2.34 (2)	2.93 (3)	1.10 (1)	
Ser		1.00 (1)		2.68 (3)		
Glx		3.25 (3)	3.35 (3)	3.04 (2)	3.18 (3)	
Gly				4.25 (4)		1.17 (1)
Ala		2.90 (3)		2.84 (2)	0.97 (1)	
Cys						
Pro			N.D. (1)	N.D. (1)		
Val			1.00 (1)	2.34 (2)	2.02 (2)	
Met				0.99 (1)	0.93 (1)	
Ile				1.04 (1)		0.93 (1)
Leu			0.99 (1)	5.00 (5)		1.00 (1)
Tyr				1.09 (1)	1.00 (1)	0.82 (1)
Phe		0.93 (1)		1.91 (2)		
Lys		1.07 (1)	1.68 (2)	6.74 (7)	1.96 (2)	2.88 (3)
His				0.90 (1)		
Arg			0.97 (1)	1.13 (1)		
Trp				N.D. (2)		
Residue no....		1–11	12–23	24–67	68–79	80–86
M_r found...		1252.0	1443.7	5041.0*	1440.6	848.6
M_r (theoretical)...		1252.3	1443.6	5024.8	1440.6	849.1

* The discrepancy between the theoretical and determined M_r for peptide 5 can be ascribed to oxidation of methionine (corrected M_r 5025.0).

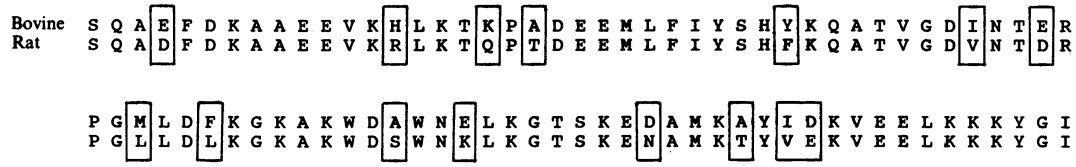

Fig. 4. Comparison of rat and bovine liver ACBP

The *N*-terminal serine residue is acetylated in both sequences (one-letter code). Non-identical residues are boxed.

than C_6 bound with a high affinity (Fig. 5). The relative affinity for different chain lengths was calculated as the slope of percentage quenching plotted against acyl-CoA concentration (Table 3). The incubations contained 37.0 μg of ACBP/ml, corresponding to a 3.71 μM. For $C_{10:0}$–$C_{16:0}$ acyl-CoA esters, inhibition of fluorescence was linear with acyl-CoA concentration up to 3.5–3.8 μM or ~ 100% saturation of ACBP. This indicates that the binding stoichiometry is 1 mol of acyl-CoA bound per mol of ACBP. These results are different from those that we reported for bovine ACBP (Mikkelsen & Knudsen, 1987), where we suggested 1 mol of acyl-CoA bound per 2 mol of ACBP. The reason for the difference is that we may have overestimated the protein content in the previous studies. In the bovine ACBP experiment, the protein was determined by weighing the freeze-dried material. In the meantime we have experienced that this is not a reliable method, probably because ACBP is highly hygroscopic. Therefore we have used amino acid analysis to determine the protein content in the present experiment. The relative affinity of rat liver ACBP for acyl-CoA of different chain lengths shows the same pattern as was observed for bovine liver ACBP (Mikkelsen & Knudsen, 1987), indicating that the two proteins have the same properties.

Bovine liver ACBP did not appear to bind fatty acid when the fluorescence assay was used. Neither could bovine ACBP be shown to bind dansyl-undecanoic acid (Mikkelsen & Knudsen, 1987). To test the ability of rat ACBP to bind fatty acids, we compared the binding of [1-^{14}C]palmitic acid and [1-^{14}C]palmitoyl-CoA in the Lipidex partition-binding assay (Burrier *et al.*, 1987). The result (not shown) clearly showed that ACBP efficiently binds palmitoyl-CoA, but is unable to bind free palmitic acid. The binding assays therefore show that, athough a medium to long hydrophobic chain is necessary for binding, it is not sufficient; the CoA part of acyl-CoA esters must also be involved in binding.

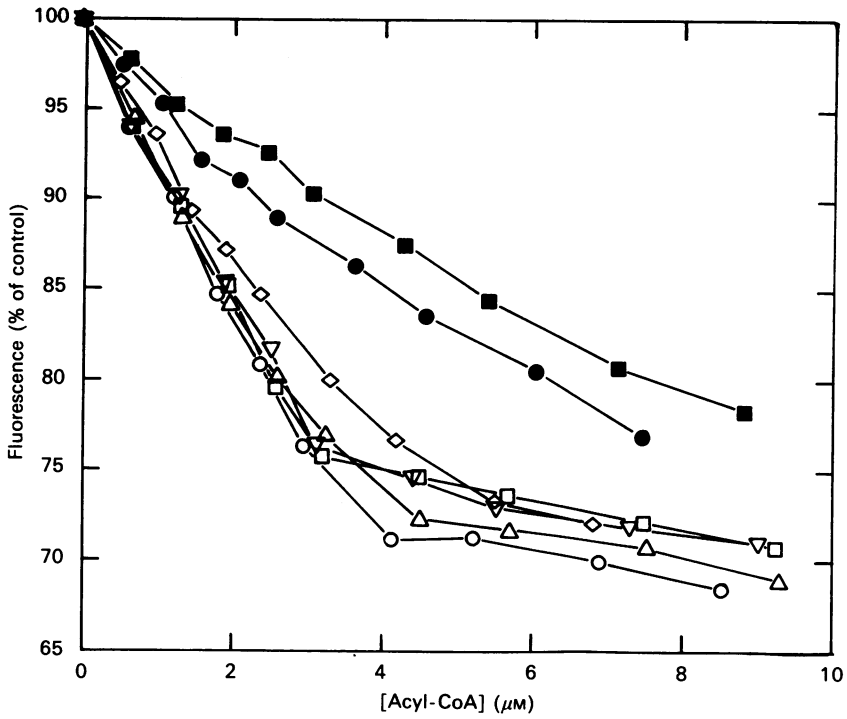

Fig. 5. Acyl-CoA binding to ACBP measured as fluorescence quenching

ACBP (37.0 μg/ml) in 10 mM-potassium phosphate buffer, pH 7.0, was excited at 280 nm, and fluorescence was measured at 332 nm in the absence and presence of different concentrations of acyl-CoA: ■, acetyl-CoA; ●, butyryl-CoA; ◇, octanoyl-CoA; △, decanoyl-CoA; ▽, dodecanoyl-CoA; ○, tetradecanoyl-CoA; □, hexadecanoyl-CoA. The results show fluorescence expressed as a percentage of that in controls without acyl-CoA esters added. For details see the Materials and methods section. The results are means of duplicates. Half the difference between duplicates did not exceed 1%.

Table 3. Relative affinity of ACBP for different chain-length acyl-CoA esters, calculated as slopes from plots of percentage of fluorescence inhibition versus acyl-CoA concentration

The data used for calculation are taken from Fig. 5.

Acyl-CoA	Slope (% inhibition of fluorescence · μM^{-1})
Acetyl-CoA	3.71
Butyryl-CoA	4.38
Octanoyl-CoA	6.67
Decanoyl-CoA	8.40
Dodecanoyl-CoA	8.25
Tetradecanoyl-CoA	8.83
Hexadecanoyl-CoA	8.06

Table 4. Subcellular distribution of ACBP in rat liver

Subcellular fractionation and e.l.i.s.a. were performed as described in the Materials and methods section. The results are means of four determinations ± S.D.

Fraction	ACBP (μg/mg of protein)	ACBP (% of total)
Nuclei (600 *g*)	1.26 ± 0.04	6.3
Mitochondria (4000 *g*)	1.60 ± 0.08	2.0
Peroxisomes plus lysosomes (25000 *g*)	1.24 ± 0.04	0.9
Microsomal fraction (100000 *g*)	2.48 ± 0.12	4.0
100000 *g* supernatant	5.68 ± 0.19	86.8

Immunological similarity of cow and rat liver ACBP

As both amino acid composition and peptide mapping show that bovine and rat liver ACBPs are closely related, one would expect that an antibody prepared against one of the proteins would recognize the other protein; however, this was not the case. Antibody raised against cow liver did not recognize rat liver ACBP, and vice versa in the e.l.i.s.a assay (results not shown). This indicates that both bovine and rat ACBP must be closely related to ACBP in rabbit.

Intracellular distribution and tissue content of ACBP

The content of ACBP in subcellular fractions obtained by differential centrifugation was determined by quantitative e.l.i.s.a. About 87% of the ACBP in rat liver was located in the soluble protein fraction (Table 4). Similar results were obtained with cow liver (Mikkelsen & Knudsen, 1987). The concentration in other tissues measured was in the same range in the rat as previously found in the cow (Table 5). ACBP in rat liver amounts to 0.64% of soluble protein, and the concentration is

Table 5. ACBP content in the 10000 *g* fraction of different tissues

Extracts from various rat tissues were made and analysed by e.l.i.s.a. as described in the Materials and methods section. The results are means of duplicates ± half the difference.

	ACBP (μg/mg of protein)	
	Rat	Cow*
Liver	6.35 ± 0.14	3.3 ± 0.5
Heart	0.53 ± 0.07	1.2 ± 0.0
Kidney	1.34 ± 0.20	1.4 ± 0.1
Muscle	0.36 ± 0.02	0.6 ± 0.07
Intestine	0.57 ± 0.02	1.5 ± 0.1
Erythrocytes (packed cells) (μg/ml)	12.1 ± 0.23	
Plasma (μg/ml)	0.08 ± 0.02	

* Mikkelsen & Knudsen (1987).

28 nmol/g of liver or 408 nmol/g of liver protein. Long-chain acyl-CoA concentration in rat liver has been reported to be 108–248 nmol/g of protein (Woldegiorgis *et al.*, 1985). The ACBP concentration is therefore about 2–4-fold higher than the long-chain acyl-CoA concentration and about 5-fold lower than the fatty acid-binding protein concentration (Wilkinson & Wilton, 1987). The importance of ACBP in binding and transport of acyl-CoA *in vivo* will depend on the relative affinity of ACBP compared with other proteins and membranes for acyl-CoAs. A possible physiological function of ACBP in acyl-CoA ester metabolism is therefore an open question until these matters have been resolved.

Identity of ACBP with diazepam-binding inhibitor

After this paper had been refereed, one of the reviewers, Dr. D. R. Headon, Department of Biochemistry, University College Galway, Galway, Ireland, informed us that ACBP was similar to or identical with diazepam-binding inhibitor (DBI). DBI, also called endozepine (Shoyab *et al.*, 1986), was first isolated from rat brain (Guidotti *et al.*, 1983) and was identified by its ability to displace diazepam competitively from the benzodiazepine-binding site on the γ-aminobutyric acid receptor from brain.

DBI has been suggested to be a putative endogenous neuropeptide acting on the benzodiazepine-receptor site on the γ-aminobutyric acid-receptor complex (Guidotti *et al.*, 1983).

The primary structure of DBI has been determined both at the cDNA level and by protein sequencing for human brain DBI (Gray *et al.*, 1986; Gray, 1987; Webb *et al.*, 1987; Marquardt *et al.*, 1986) and bovine brain DBI (Webb *et al.*, 1987; Marquardt *et al.*, 1986) and on the cDNA level for rat brain DBI (Mocchetti *et al.*, 1986; Gray, 1987). The sequence of rat and bovine brain DBI is completely identical with that of rat liver ACBP reported in the present paper and that of bovine liver ACBP (Mikkelsen *et al.*, 1987) respectively.

The identity of DBI and ACBP raises the question: is DBI a neurotransmitter or a lipid-transport protein, or both? Both measurement of ACBP levels by e.l.i.s.a. (Mikkelsen & Knudsen, 1987) and determination of DBI-gene expression (Alho *et al.*, 1988) show that ACBP/DBI is found in all tissues investigated. The highest concentration of ACBP in the soluble protein fraction was found in liver (Mikkelsen & Knudsen, 1987). The real physiological function of ACBP/DBI will have to await complete exploration of the mechanism of DBI interaction with the GABA-receptor benzodiazepine-binding site and the ability of ACBP to act as a carrier of acyl-CoA and other lipophilic ions.

This work was supported by the Carlsberg Foundation and the Danish Natural Science Research Council. We thank Mr. Erling Knudsen and Ms. Annelise V. Andersen for excellent technical assistance and Lisbeth Johansen for typing the manuscript.

REFERENCES

Alho, H., Fremeau, R. T., Jr., Tiedge, H., Wilcox, J., Bowolin, P., Brosius, J., Roberts, J. L. & Costa, E. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7018–7022

Burrier, R. E., Manson, C. R. & Brecher, P. (1987) Biochim. Biophys. Acta **919**, 221–230

Gray, P. W. (1987) Neuropharmacology **26**, 863–865

Gray, P. W., Glaister, D., Seeburg, P., Guidotti, A. & Costa, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7547–7551

Guidotti, A., Forchetti, C. M., Konkel, D., Bennet, C. D. & Costa, E. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 3531–3535

Haunerland, N., Jagschies, G., Schulenberg, H. & Spener, F. (1984) Hoppe-Seyler's Z. Physiol. Chem. **365**, 365–376

Hunkapiller, M. W. & Hood, L. F (1983) Methods Enzymol. **91**, 485–493

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

Marquardt, H., Todaro, G. J. & Shoyab, M. (1986) J. Biol. Chem. **261**, 9727–9731

Mikkelsen, J. & Knudsen, J. (1987) Biochem. J. **248**, 709–714

Mikkelsen, J., Højrup, P., Nielsen, P. F., Roepstorff, P. & Knudsen, J. (1987) Biochem. J. **245**, 857–861

Mocchetti, L., Einstein, R. & Brosins, J. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7221–7225

Mogensen, I. B., Schulenberg, H., Hansen, H. O., Spener, F. & Knudsen, J. (1987) Biochem. J. **241**, 189–192

Nielsen, P. F., Klarskov, K., Højrup, P. & Roepstorff, P. (1988) Biomed. Environ. Mass. Spectrom. **17**, 355–362

Pawlak, J. & Smith, S. (1986) Cancer Res. **46**, 4712–4719

Roepstorff, P. (1978) Biomed. Mass Spectrom. **5**, 362–366

Shoyab, M., Gentry, L. E., Marquardt, H. & Todaro, G. J. (1986) J. Biol. Chem. **261**, 11968–11973

Tanaka, T., Slamon, D. J. & Cline, M. J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 3400–3404

Webb, N. R., Rose, T. M., Malik, N., Marquardt, H., Shoyab, M., Todaro, G. & Lee, D. (1987) DNA **6**, 71–79

Wilkinson, T. C. I. & Wilton, D. C (1987) Biochem. J. **242**, 913–917

Woldegiorgis, G., Spennetta, T., Corkey, B. E., Williamson, J. R. & Shrago, E. (1985) Anal. Biochem. **150**, 8–12

Received 30 December 1988/11 April 1989; accepted 28 April 1989