Long-chain acyl-CoA esters and acyl-CoA binding protein are present in the nucleus of rat liver cells

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Abstract A detailed analysis of the subcellular distribution of acyl-CoA esters in rat liver revealed that significant amounts of long-chain acyl-CoA esters are present in highly purified nuclei. No contamination of microsomal or mitochondrial marker enzymes was detectable in the nuclear fraction. C16:1 and C18:3-CoA esters were the most abundant species, and thus, the composition of acyl-CoA esters in the nuclear fraction deviates notably from the overall composition of acyl-CoA esters in the cell. After intravenous administration of the non-β-oxidizable [^{14}C]tetradecylthioacetic acid (TTA), the TTA-CoA ester could be recovered from the nuclear fraction. Acyl-CoA esters bind with high affinity to the ubiquitously expressed acyl-CoA binding protein (ACBP), and several lines of evidence suggest that ACBP functions as a pool former and transporter of acyl-CoA esters in the cytoplasm. By using immunohistochemistry, immunofluorescence microscopy, and immunoelectron microscopy we demonstrate that ACBP localizes to the nucleus as well as the cytoplasm of rat liver cell and rat hepatoma cells, suggesting that ACBP may also be involved in regulation of acyl-CoA-dependent processes in the nucleus.—Elholm, M., A. Garras, S. Neve, D. Tornehave, T. B. Lund, J. Skorve, T. Flatmark, K. Kristiansen, and R. K. Berge. **Long-chain acyl-CoA esters and acyl-CoA binding protein are present in the nucleus of rat liver cells.** ***J. Lipid Res.*** **2000.** 41: **538–545.**

Supplementary key words long-chain acyl-CoA • acyl-CoA binding protein • nuclear localization • tetradecylthioacetic acid.

Long-chain acyl-CoA esters are versatile molecules that are involved in a plethora of cellular functions. Apart from being necessary substrates for acylation reactions, acyl-CoA esters have beeen reported to be key regulators of fatty acid synthesis and serve as regulators of various other enzyme systems, vesicle trafficking, ion channels, and ion pumps (reviewed in 1–4). Long-chain acyl-CoA esters are subject to elongation and desaturation, and undergo β-oxidation in peroxisomes and mitochondria (1). It is well established that long-chain acyl-CoA esters are involved in the regulation of gene expression in prokaryotes (5, 6) and yeast (7), and recently it was reported that HNF-4α-mediated transactivation was enhanced upon binding of certain acyl-CoA esters (8).

Due to limited solubility and ubiquitous thioesterase activity within the cell, the biological functions of acyl-CoA esters may to a large extent depend on carrier proteins able to transport and protect the acyl-CoA esters against hydrolysis. One such protein is the acyl-CoA binding protein (ACBP), a highly conserved 10 kDa protein which binds acyl-CoA esters with a K_D in the low nm range (9, 10), but yet ACBP is able to deliver acyl-CoA to acyl-CoA-dependent biological systems (10). ACBP is expressed in a wide variety of species ranging from yeast to mammals and is found to be expressed in all tissues examined (2). Traditionally, ACBP has been considered as a cytosolic protein (11, 12).

The presence of fatty acid Δ5-desaturase (13) and long-chain acyl-CoA synthetase activity (14) in rat liver cell nuclei strongly suggest that a pool of long-chain acyl-CoA esters should exist in the nucleus. In the present study we have performed a more detailed analysis of the subcellular distribution of long-chain acyl-CoA esters in rat liver. We demonstrate that highly purified nuclei contain significant amounts of long-chain acyl-CoA esters, most notably C16:1 and C18:3 CoA esters. In addition, we present evidence that ACBP is not only present in the cytoplasm but also exhibits a prominent nuclear localization in rat liver and rat hepatoma cells.

Abbreviations: ACBP, acyl-CoA binding protein; ALPB, adipocyte lipid binding protein/aP2; HNF-4α, hepatocyte nuclear ractor 4α; KLBP, keratinocyte lipid binding protein; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; RXR, retinoid X receptor; TTA, tetradecylthioacetic acid.

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EXPERIMENTAL PROCEDURES

Chemicals

p-Hydroxymercuribenzoic acid sodium chloride (PHMB), 5,5′-dithio-bis(2-nitrobenzoic acid) (DNTB), palmitoyl-coenzyme A (16:0), heptadecanoyl-coenzyme A (17:0), palmitoleoyl-coenzyme A (16:1), oleoyl-coenzyme A (18:1), linoleoyl-coenzyme A (18:2), linolenoyl-coenzyme A (18:3), and arachidonoyl-coenzyme A (20:4) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were obtained from common commercial sources and were of analytical grade.

Synthesis of [1-^{14}C]tetradecylthioacetic acid

Prior to use, 1-tetradecanethiol was distilled at 84–86°C/0.01 mm Hg and [^{14}C]bromoacetic acid (1 mCi bromoacetic acid (55 mCi/mmol)) was distilled at 117–118°C/15 mm Hg. All other chemicals were flushed with argon. 1-Tetradecanethiol (42 mg) was placed in a round-bottom flask. Two ml methanol was added and the mixture was stirred under argon to prevent oxidation of the sulfides. After 15 min, 1.70 ml of 0.75 m KOH in methanol was carefully added. The reaction mixture was then stirred for 20 min. Nineteen mg [^{14}C]bromoacetic acid was dissolved in 10 ml methanol. The solution was allowed to evaporate to 5 ml under a flush of nitrogen after which 1.68 ml of 0.757 m KOH was added. The reaction mixture was boiled for 24 h with a continuous flow of argon into the mixture. Forty-eight mg of 37% HCl in 10 ml water was then added. This led to precipitation of the product. Before the mixture was set at 4°C to complete precipitation, pH was measured and, if necessary, adjusted to a value between 4 and 5. [1-^{14}C]tetradecylthioacetic acid was transferred to a G-4 filter, washed with distilled water, and recrystallized from dichloromethane in 10% (w/w) pentane. It had a melting point of 63°C and the yield was 78%.

Animal care and treatment

Pathogen-free male Wistar rats (weighing 150–200 g) and adult Sprague-Dawley rats used for immunohistochemistry and immunoelectron microscopy were obtained from Møllegaard Breeding laboratory (Ejby, Denmark), housed as pairs, and maintained on a 12 h light–dark cycle at 20 ± 3°C. Standard pellet and water were available ad libitum. The Wistar rats were acclimatized under these conditions for 1 week before beginning the experiments. After 20 min at 30°C, rats were lightly anesthetized with halothane, and each rat was injected with 0.5 ml of the [^{14}C]tetradecylthioacetic acid solution (6.5 mCi/mmol, 20 μCi/ml in 0.9% (w/v) NaCl, 5% (w/v) BSA) through the tail vein. Rats were killed by cardiac puncture. Six ml blood was collected in EDTA vacutainers and a part of the liver was removed and freeze-clamped for later extraction of total acyl-CoA esters. Eight ml High Flash Point™, Universal LSC-cocktail (Packard Instrument Company, Meriden, CT) was added to the 50–100 μl homogenized tissue, and the radioactivity was determined in a liquid scintillation spectrometer. The local ethical committee for animal experiments approved the use of the animals.

Preparation of nuclear fractions and liver homogenate for solid phase extraction of long-chain acyl-CoA esters

Rat livers, 6–8 g, were minced and a nuclear fraction was prepared as described by Gorski, Carneiro, and Schibler (15) except that the centrifugation was performed at 21,000 rpm for 20 min, the second purification was omitted, and 0.4 mm PHMB was added to the homogenization buffer. Freeze-clamped rat liver, 0.5 g, was homogenized in 100 mm KH_2PO_4 buffer pH 4.9. Solid phase extraction of long-chain acyl-CoA was performed on both liver homogenate and the nuclear fraction as described by Deutsch et al. (16) except that the centrifugation was done at 14,000 rpm for 8 min.

Identification of long-chain acyl-CoAs by HPLC

Long-chain acyl-CoA esters were separated by HPLC according to Rosendal and Knudsen (17). One hundred and fifty μl of the extracts was injected onto a 4.6 × 250 mm column packed with Nucleosil ODS (C_{18}), 10-μm particle size and 100 Å pore size. Absorbance was measured at 254 nm. Elution solvent A was 20% (v/v) acetonitril and 80% (v/v) 25 mm KH_2PO_4, pH 5.3, and solvent B was 70% (v/v) acetonitril and 30% (v/v) 25 mm KH_2PO_4, pH 5.3. The elution was carried out at room temperature with a flow rate of 1 ml/min. The profile of the gradient (buffer A in B) was as follows: 85% A for 15 min, 85% A to 75% A in 5 min, 75% A for 15 min, 75% A to 50% A in 55 min, 50% A for 5 min, 50% A to 15% A in 5 min, 15% A for 5 min, 15% A to 0% A in 5 min, 0% A for 15 min. Baseline was reestablished after 15 min of washing with 85% A. One-ml fractions were collected and the radioactivity was quantitated in a liquid scintillation spectrometer. The buffers were filtrated and degassed before use and were used with continuous stirring.

Heptane/water extraction of acyl-CoA esters

To 300 μl nuclear extract of acyl-CoA esters were added 1 ml of water-saturated heptane and 1 ml of heptane-saturated water and the mixture was vigorously shaken for 10 min. The radioactivity in the upper heptane layer containing fatty acids and the lower aqueous layer containing acyl-CoA esters was measured in a liquid scintillation spectrometer.

Enzyme assay

The NADPH cytochrome *c* reductase was measured according to Sottocasa et al. (18) and the glutamate dehydrogenase was measured with the GlDH kit (Boehringer Mannheim) according to the instruction. The long-chain acyl-CoA thioesterase activity was assayed according to Berge (19).

Two-dimensional polyacrylamide gel electrophoresis and Western blotting

Two-dimensional polyacrylamide gel electrophoresis was performed according to O'Farrell (20). Western blotting was performed as described previously (21).

Fixation of rat tissues

Adult Sprague-Dawley male rats (n = 7) were anesthetized with Nembutal. The thorax cavity was opened and the animals were perfused through the left ventricle with 300 ml physiological saline buffer containing 0.0005% (w/v) heparin followed by 450 ml 4% (w/v) paraformaldehyde or 0.1% (v/v) glutaraldehyde in phosphate-buffered saline, 137 mm NaCl, 2.7 mm KCl, 4.3 mm $Na_2HPO_4 \cdot 7H_2O$, KH_2PO_4, pH 7.3 (PBS). For immunohistochemical examinations, fixation was performed in 4% (w/v) paraformaldehyde for 4 h at 4°C and, for immunoelectron microscopy, fixation was performed in 0.1% (v/v) glutaraldehyde for 1 h at 4°C.

Imunohistochemistry

Immunohistochemical analyses were performed as described previously (22) except that blocking of endogenous peroxidase activity with HIO_4 and quenching of residual aldehyde groups with $NaBH_4$ were omitted.

Immunoelectron microscopy

The fixed tissue was embedded in 1.25% (w/v) melted agar. Agar blocks (1 × 1 mm) were dehydrated in graded series of ethanol equilibrated at progressively lower temperatures and infiltrated with lowicrylR resin K4M (Chemische Werke Lowi, Waldkraiburg) at 35°C. Ultrathin sections (500–600 Å) were placed on Formvar-coated nickel grids and used for on-grid immu-

nogold staining as described previously (21). In brief, the grids were incubated in blocking solution containing 0.8% (w/v) BSA, 0.1% (v/v) quality gelatin, 5% (v/v) normal goat serum, and 2 mm NaN_3 in PBS and then incubated overnight at 4°C, with rabbit anti-ACBP anti-serum diluted 1:50–1:200. After several washes the grids were incubated overnight at 4°C with 5 nm gold-conjugated goat anti-rabbit IgG (Sigma, MO) diluted 1:25. The antibodies were diluted in blocking solution containing 1% goat serum. Controls were performed by omission of the primary antiserum. After postfixation in 2% (v/v) glutaraldehyde, the grids were poststained with uranyl acetate and lead citrate and examined in a Zeiss 902 electron microscope.

Cell culture and transfection

H4-IIE-C3 rat hepatoma cells (ATCC CRL-1600) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum in a humidified atmosphere of 5% CO_2 at 37°C. COS-7 cells (ATCC CRL-1651) were cultured in RPMI 1640 medium comtaining 10% (v/v) fetal calf serum. Plasmids were purified by using a Midi Preparation kit (Qiagen). Transfection of COS-7 cells was accomplished using the calcium phosphate co-precipitation method. Briefly, 10% confluent cells in 10 cm^2 slide flasks (plated 24 h prior to transfection) were transfected with 6 μg of supercoiled pCMV-rACBP plasmid (23). The calcium phosphate DNA precipitate was left on the cells for 16 h and the cells were analyzed for ACBP expression 2 days post transfection by in situ staining.

In situ immuno-staining of transfected COS-7 cells and rat H4-IIE-C3 hepatoma cells

The cells were rinsed in ice-cold PBS and fixed in 4% (v/v) paraformaldehyde in PBS at 4°C for 5 min. The cells were washed three times in ice-cold PBS and permeabilized with 70% ethanol for 5 min at −20°C. The cells were washed in PBS and incubated for 1 h at room temperature with a 1:50 dilution of affinity-purified rabbit polyclonal anti-rat ACBP antibodies in 1% (w/v) BSA in PBS. Subsequently, the cells were washed with PBS and incubated with 0.85 μg/ml fluorescein-conjugated porcine anti-rabbit secondary antibody (Dakopatts) in 1% (w/v) BSA in PBS for 1 h at room temperature. Nuclear DNA was counterstained with 1 μg/ml Hoechst 33258 in PBS, and slides were evaluated by standard UV-fluorescence microscopy.

RESULTS

Identification of acyl-CoA in rat liver homogenates and in rat liver nuclear fraction

In order to investigate whether a nuclear pool of long-chain acyl-CoA ester exists, 6–8 g of liver was homogenized and a nuclear fraction was prepared according to Gorski et al. (15). This protocol allows isolation of nuclei of high purity. To detect whether the isolated nuclei were contaminated with mitochondria and/or microsomal membranes, the enzymatic activities of glutamate dehydrogenase and NADPH cytochrome *c* reductase were determined in the nuclear fraction and the postnuclear supernatant. The enzymatic activities of glutamate dehydrogenase and NADPH cytochrome *c* reductase in the postnuclear supernatant were 340 nmol/min per mg protein and 3.73 nmol/min per mg protein, respectively, whereas the enzymatic activity in the nuclear fraction was below the detection limits making a possible contamination of the nuclear fraction negligible (results not shown).

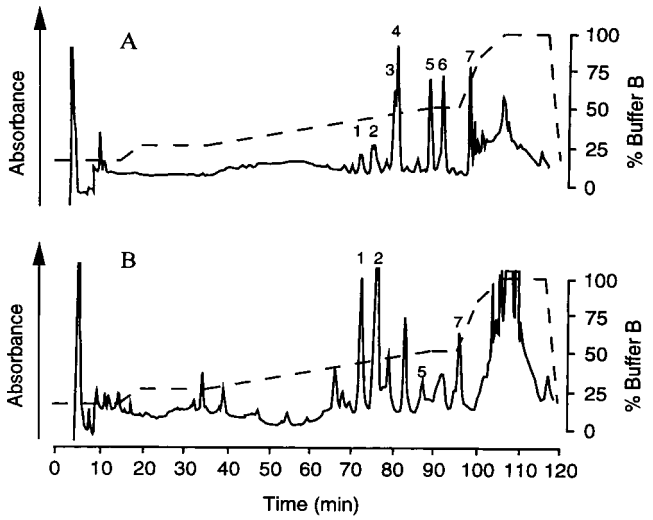

Fig. 1. HPLC chromatogram of total acyl-CoA in liver homogenate (A) and total acyl-CoA in the nuclear fraction (B). The individual esters were identified on the basis of their retention time relative to that of heptadecanoyl-CoA added as an internal standard; (peak/ester), (1/18:3), (2/16:1), (3/20:4), (4/18:2), (5/16:0 and TTA), (6/18:1), (7/17:0). Absorbance at 254 nm (solid line) and percent buffer B (dashed line).

Acyl-CoA esters were extracted from the purified nuclei and from a total liver homogenate by solid phase extraction of long-chain acyl-CoAs according to Deutsch *et al.* (16). **Figure 1A** shows a typical HPLC profile of acyl-CoA esters extracted from the rat liver homogenate. Heptadecanoyl-CoA was used as an internal standard for calculation of relative retention times (RRT) for standard long-chain acyl-CoA esters (**Table 1**). From the relative retention times, C20:4, C18:2, C18:1, and C16:0 were found to be the most abundant acyl-CoA esters in the liver homogenate. This profile is in agreement with previously published results (16, 17).

Figure 1B shows a typical HPLC profile of acyl-CoA esters extracted from isolated nuclei. The HPLC profiles of nuclear and total liver acyl-CoA esters were clearly distinct in that the profile of acyl-CoA esters extracted from the nuclei contained predominantly C18:3 and C16:1 acyl-CoA

TABLE 1. Retention time (RT) and relative retention time (RRT) for selected long-chain acyl-CoAs using heptadecanoyl-CoA as an internal standard

	n	RT	$RRT_{17:0}$
		min	
16:0	3	80.10 ± 2.23	0.895 ± 0.003
16:1	5	65.75 ± 2.23	0.736 ± 0.011
17:0	31	88.58 ± 1.53	1
18:1	5	82.09 ± 1.09	0.929 ± 0.003
18:2	5	71.05 ± 1.36	0.805 ± 0.001
18:3	5	61.76 ± 1.21	0.699 ± 0.002
20:4	8	70.52 ± 1.22	0.796 ± 0.001

Long-chain acyl-CoA esters were mixed with heptadecanoyl-CoA and separated by HPLC. The values represent the means ± SD, where n is the number of HPLC runs.

esters. Because microsomal and mitochondrial marker enzyme activities could not be detected in the nuclear fraction, it is unlikely that the specific subset of acyl-CoA esters found in the nuclear fraction originates from contaminating microsomal membranes or mitochondria. By using a microsomal fraction as the source of thioesterase activity and following the release of CoA with DNTB (24), the nuclear pool of long-chain acyl-CoA esters was estimated to be 0.32 nmol/g wet liver (result not shown).

In order to examine the efficiency of the extraction protocol we examined whether it was possible to extract the CoA ester of a fatty acid administrated intravenously to the rat. Radiolabeled [1-^{14}C]tetradecylthioacetic acid was injected through the tail vein and the rats were killed 1, 20, 40, and 90 min after injection. The plasma was collected and the radioactivity was measured. Clearance of tetradecylthioacetic acid from the plasma occurred within the first minutes (**Table 2**). The liver, heart, epididymal fat, and brain were removed, freeze clamped, homogenized, and the radioactivity present in the homogenates was determined. We found that most of the radioactivity was recovered in the liver homogenates at all time points (Table 2).

Tetradecylthioacetic acid is activated to its CoA ester in vivo (17). To examine whether [1-^{14}C]tetradecylthioacetyl-CoA was present in the total liver homogenate and the nuclear fraction, the extracted acyl-CoA esters were separated by HPLC and the radioactivity in the eluate was determined. **Figure 2A** shows the profile of radioactivity in the eluate of total liver acyl-CoA esters. The bulk of the radioactivity was found in the fraction in which tetradecylthioacetyl-CoA eluted. In the eluate of nuclear acyl-CoA esters, most of the radioactivity was found in the fraction containing tetradecylthioacetyl-CoA, but a significant amount was also found in a fraction eluting after 21 min (Fig. 2B). The identity of this metabolite remains to be established. It is known that tetradecylthioacetic acid can be metabolized via the cytochrome P450 hydroxylase (25), the flavin monooxygenase (26, 27), and the Δ9-desaturase (28), and the resulting metabolites would be expected to elute earlier under more hydrophilic condition than tetradecylthioacetyl-CoA. The amount of [^{14}C]acyl-CoA recovered from the nuclear fraction constituted consistently 3–5% of the total amount of [^{14}C]acyl-CoA in the whole cell liver homogenate (n = 12, result not shown).

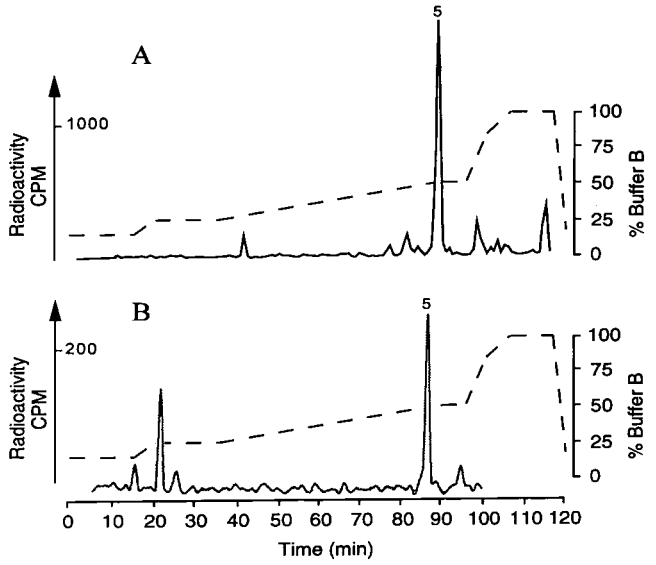

Fig. 2. Recovery of ^{14}C-labeled acyl-CoA esters in total liver homogenates and nuclei isolated from rats injected with [1-^{14}C]tetradecylthioacetic acid. Total acyl-CoA esters were extracted from total liver homogenates (A) and isolated nuclei (B) and fractionated by HPLC. Radioactivity in the collected fractions was determined by liquid scintillation counting. Peak no. 5 elutes with the same retention time as authentic tetradecylthioacetic acid. Radioactivity (solid line) and percent buffer B (dashed line). For details see text.

To further substantiate the finding that [1-^{14}C]tetradecylthioacetyl-CoA is present in the nucleus, the acyl-CoA esters extracted from nuclei were extracted with heptane/water with or without prior alkaline hydrolysis. **Table 3** shows that 73% of the radioactivity was recovered in the aqueous phase in the absence of treatment with NaOH, whereas 85% of the radioactivity was recovered in the heptane phase after alkaline hydrolysis.

Subcellular distribution of the acyl-CoA binding protein (ACBP) in rat liver and COS-7 cells

ACBP has been shown to act as an intracellular acyl-CoA pool former and transporter (10, 29). Thus, the presence of long-chain acyl-CoA esters in the nucleus warranted a more thorough analysis of the intracellular localization of

TABLE 2. Organ distribution of [^{14}C]tetradecylthioacetic acid as a function of time after adminstration

Time	Plasma (10^{-6})	Liver	Heart	Brain	Epididymal Fat
min	*cpm*	*cpm/mg protein*			
1	3.09 ± 0.21	9212 ± 1862	1420 ± 263	853 ± 70	1711 ± 466
20	0.52 ± 0.14	9871 ± 1693	600 ± 151	1038 ± 356	1655 ± 202
40	0.83 ± 0.14	10398 ± 2555	400 ± 118	1136 ± 443	2024 ± 390
90	0.51 ± 0.15	12710 ± 2358	319 ± 55	737 ± 275	3380 ± 838

[^{14}C]tetradecylthioacetic acid was administrated intravenously to the rats, and plasma and tissues were collected and analyzed as described in Experimental Procedures. The values represent means ± SD of three animals in each group and are given as cpm/mg protein for the tissues and cpm for total plasma volume.

TABLE 3. Heptane/water extraction of the acyl-CoA esters extracted from isolated nuclei

	−NaOH		+NaOH	
	cpm	% of total	cpm	% of total
Water phase	418	73	49	15
Heptane phase	147	27	286	85
Recovery	100%		117%	

Two fractions of acyl-Coa esters extracted from nuclei were subjected to extraction with heptane/water with or without prior treatment with NaOH. Radioactivity in each phase was determined as described in Experimental Procedures.

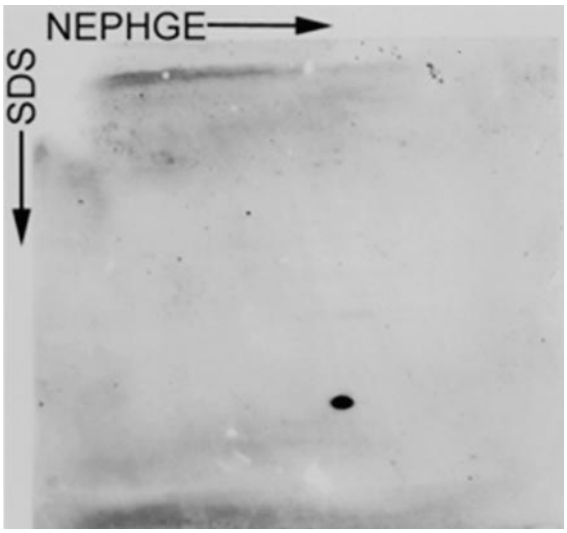

Fig. 3. Specificity of affinity-purified anti-rat ACBP antibodies. Western blot analysis of rat liver proteins separated by two-dimensional polyacrylamide gel electrophoresis. The blot was incubated with affinity-purified anti-rat ACBP antibodies followed by incubation with a secondary peroxidase-conjugated antibody and detection by ECL.

ACBP. ACBP has generally been considered as a cytoplasmic protein, although early fractionation studies suggested that ACBP was also present in the nuclear fraction (11, 12).

Affinity-purified antibodies against rat ACBP were prepared. The specificity of the antibody preparation was ascertained by Western blotting of rat liver proteins separated by two-dimensional polyacrylamide gel electrophoresis. **Figure 3** shows that the antibodies recognized only one spot with the expected molecular mass and p*I* on the two-dimensional gels. This antibody preparation was used for immunohistochemical analyses of sections of rat liver cells. As expected, strong ACBP immunoreactivity was observed throughout the sections. The cytoplasm exhibited strong staining, but in addition, staining of nuclei was clearly visible (**Fig. 4**). More detailed analyses were performed by immunoelectron microscopy. **Figure 5A** shows an overview of a liver section dominated by the nucleus, and Fig. 5B shows an enlargement of a section of the nucleus. Numerous gold particles are present over the nucleus substantiating the results of the immunohistochemical analysis. Control sections processed with no anti ACBP antibodies were devoid of gold particles (not shown). Determination of intracellular localization by immunoelectron microscopy is prone to fixation artifacts. Consequently, we decided to study the intracellular localization of ACBP in H4-IIE-C3 rat hepatoma cells which express high levels of ACBP. In keeping with the localization studies of rat liver, immunofluorescence analysis revealed the presence of ACBP in the cytoplasm as well as in the nucleus of the hepatoma cells (**Fig. 6**). Finally, we analyzed the intracellular localization of ACBP expressed ectopically in COS-7 cells. ACBP expression is low in COS-7 cells, and as shown in **Fig. 7A**, untransfected cells exhibit no ACBP immunoreactivity using the affinity-purified anti

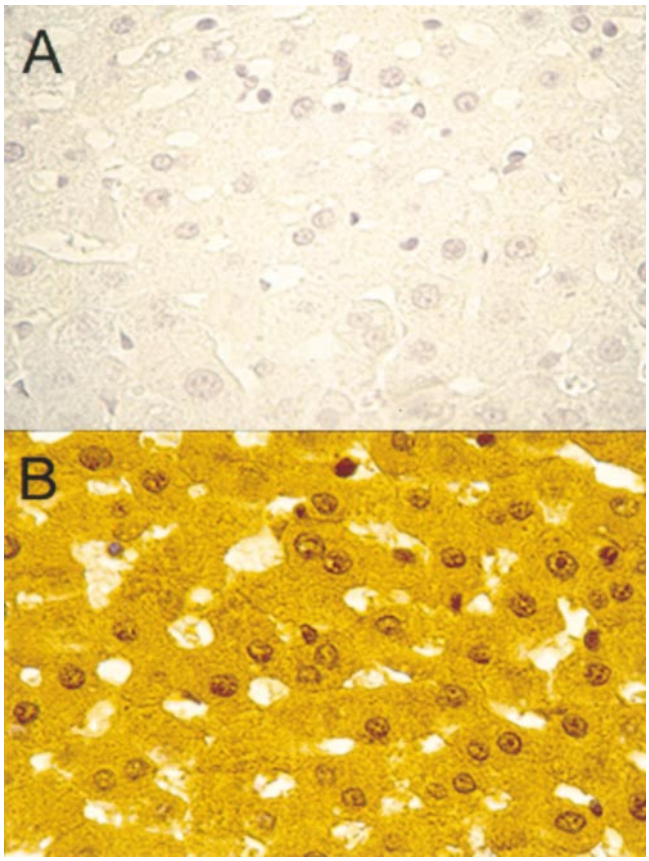

Fig. 4. Immunohistochemical analysis of ACBP localization in rat liver. (A) Control staining with omission of the primary antibody. (B) Localization of ACBP in rat liver as revealed by staining with affinity-purified rabbit antirat ACBP antibodies. ACBP immunoreactivity is uniformly distributed throughout the liver with strong signals in hepatocytes. Note the equal staining of the cytoplasm and the nuclei.

rat ACBP antibodies. Transfection with the ACBP expressing vector resulted in clear labeling of numerous cells and, interestingly, ACBP immunoreactivity is very prominent in the nuclei (Fig. 7B–D). Thus, ACBP is clearly able to localize to the nucleus, and may even under special conditions be primarily localized in this compartment of the cell.

DISCUSSION

In the present work we provide evidence that a significant pool of long-chain acyl-CoA esters is present in the nuclei of rat liver. In addition, we show that intravenously administrated [1-^{14}C]tetradecylthioacetic acid rapidly is taken up by the liver and converted into [1-^{14}C]tetradecylthioacetyl-CoA that can be recovered in whole liver homogenates as well as in highly purified nuclei (Fig. 1). Interestingly, our results revealed that the chain length composition of the nuclear acyl-CoA pool is different from that of the total liver homogenates. C16:1 and C18:3 CoA esters are predominantly found in the nuclear fraction, whereas C20:4, C18:1, C18:2, and C16:0 acyl-CoA esters

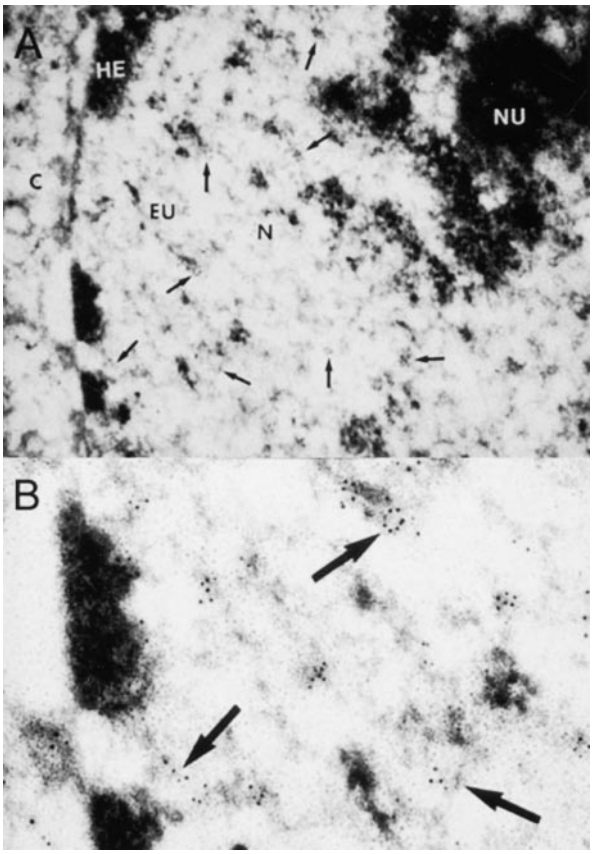

Fig. 5. Examination of the intracellular localization of ACBP in rat hepatocytes by immunoelectron microscopy. A section of rat liver was examined as described in Experimental Procedures. (A) Low magnification of a rat liver cell. (B) Enlarged section of A. Numerous gold particles representing ACBP immunoreactivity are indicated by arrows. Cytosol (C), nucleus (N), heterochromatin (HE), euchromatin (EU), and nucleoli (NU).

characterize whole liver homogenates. While previous studies on the regulatory function of long-chain acyl-CoA esters have been focused on the overall changes in the cellular concentration of acyl-CoA esters (24, 30, 31), our results suggest that differential intracellular distribution rather than a general increase in the acyl-CoA pool may play an important role in acyl-CoA-mediated regulation.

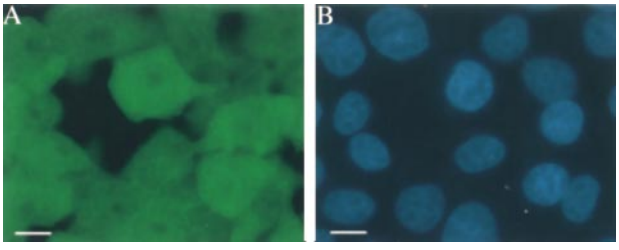

Fig. 6. Examination of the intracellular localization of ACBP in H4-IIE-C3 hepatoma cells by in situ immuno-fluorescence microscopy. (A) ACBP was detected by using affinity-purified rabbit anti-rat ACBP antibodies and visualized by a fluorescein-conjugated porcine anti-rabbit secondary antibody. ACBP immunoreactivity is uniformly distributed throughout the cell. (B) Control cells (bar 10 μm). Nuclear DNA was counterstained with Hoechst 33258.

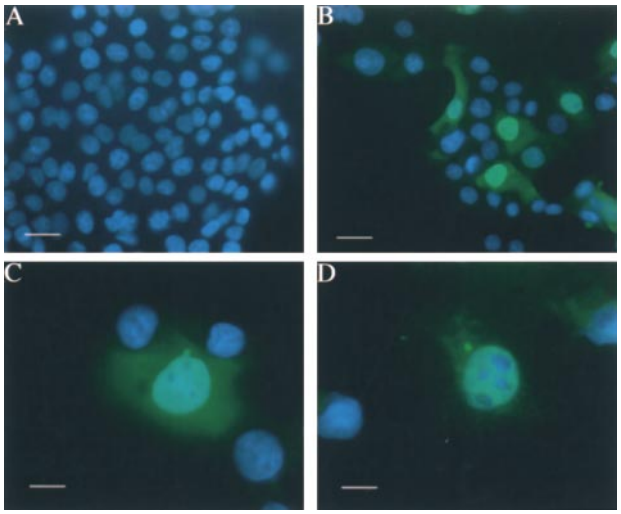

Fig. 7. Examination of the intracellular localization of ACBP in COS-7 cells expressing recombinant rat ACBP by in situ immunofluorescence microscopy. (A) Non-transfected cells. (B–D) Cells transfected with the ACBP-expressing vector. ACBP was detected with the affinity-purified rabbit anti-rat ACBP antibodies and visualized by a fluorescein-conjugated porcine anti-rabbit secondary antibody. Nuclear DNA was counter-stained with Hoechst 33258. (B) Low magnification of transfected cells (bar 25 μm). (C) and (D) enlargement of (B) (bar 10 μm). Transfected cells display staining of both the nucleus and the cytoplasm. The nuclear fluorescence is evenly distributed throughout the nucleus, except for regions corresponding to nucleoli.

Along this line it is of interest that the recently reported activation of HNF-4α by acyl-CoA esters was highly dependent on the nature of the acyl-CoA species (8).

It is well established that PPARα play a prominent role in transcriptional regulation of genes encoding fatty acid metabolizing enzymes. Numerous compounds including natural fatty acids and TTA have been shown to bind to and activate PPARα. Many of these compounds are activated to their corresponding CoA esters (32–34). However, direct evidence for a role of acyl-CoA esters in the regulation of PPARα activity has not been presented and the lack of a correlation between total acyl-CoA levels and gene expression has questioned the importance of acyl-CoA ester in PPARα-mediated transactivation (32–34). It has been suggested that activation to CoA esters represents a way to inactivate PPARα activators, and in keeping with this view it was shown that cotransfection of a vector expressing acyl-CoA synthetase compromised PPARα-mediated transactivation (35). Recent results from our laboratories show that the long-chain acyl-CoA esters in vitro may antagonize the effects of ligand induced PPAR-RXR/PPRE complex formation (M. Elholm, I. Dam, C. Jørgensen, A. Krogsdam, D. Holst, I. Kratchmarova, M. Göttlicher, J. Å. Gustafsson, R. K. Berge, T. Flatmark, J. Knudsen, S. Mandrup, and K. Kristiansen, unpublished results) implying that a nuclear pool of acyl-CoA esters may antagonize PPARα-dependent transactivation. Whether this is of biological significance remains to be established.

Interestingly, 16:1-CoA and 18:3-CoA are the predominant nuclear acyl-CoA species even though the levels of

the corresponding free fatty acids are low in nuclei (13). This is not unprecedented as other studies clearly have shown that the cellular level of long-chain acyl-CoA esters does not necessarily correlate with the level of the corresponding fatty acids (17, 36). The differential recovery of acyl-CoA esters of modified TTA, possibly comprising desaturated TTA, in the nuclei of rat liver suggests that compartment-specific metabolism may, in part, determine the profile of acyl-CoA esters. Interestingly, both C16:0 and C18:2 fatty acids are prominent in nuclei (13), and hence the abundance of C16:1 and C18:3 acyl-CoA esters suggests the presence of significant levels of Δ9-desaturase and Δ6-desaturase activity in the nuclear compartment. Differential levels of acyl-CoA hydrolyzing thioesterases and long-chain acyl-CoA synthetases with different specificities or substrate preferences may also contribute to the nuclear-specific profile of acyl-CoA esters (14, 37–40). Finally, it is noteworthy that the C18:3 acyl-CoA ester was reported to be a potent antagonist of HNF-4α-mediated transactivation (8)

The presence of acyl-CoA esters in the nucleus suggests that the high-affinity binding protein ACBP may also be localized to the nucleus. By using a combination of immunohistochemistry, immunoelectron microscopy, and conventional immunofluorescence microscopy we provide conclusive evidence that ACBP apart from being present in the cytoplasm also exhibits a prominent nuclear localization in rat liver nuclei and transfected COS-7 cells. In unpublished studies (T. Helledie, M. Antonius, R. V. Sørensen, A. V. Hertzel, D. A. Bernlohr, S. Kolvraa, S. Mandrup, and K. Kristiansen) we have shown that the lipid binding proteins ACBP as well as ALBP and KLBP localize to both the cytoplasm and nuclei in 3T3-L1 cells and transfected CV-1 cells, and that ectopic expression of lipid binding proteins modulates ligand-dependent transactivation by PPARs.

In conclusion, the results of the present study demonstrate that long-chain acyl-CoA esters and the high-affinity acyl-CoA binding protein ACBP are present in significant amounts in nuclei of rat liver hepatocytes and rat hepatoma cells. Surprisingly, the profile of long-chain acyl-CoA esters in nuclei deviates notably from that of whole cell homogenates suggesting that differential intracellular distribution of acyl-CoA esters may constitute a novel mechanism for regulating acyl-CoA-dependent processes. The nuclear localization of ACBP further suggests that ACBP in the nucleus as in the cytoplasm may function as a pool former and acyl-CoA transporter, and hence that the biological effects of acyl-CoA esters in the nucleus may also be regulated by the availability of ACBP.

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