Yeast acyl-CoA-binding protein: acyl-CoA-binding affinity and effect on intracellular acyl-CoA pool size

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Acyl-CoA-binding protein (ACBP) is a 10 kDa protein characterized in vertebrates. We have isolated two ACBP homologues from the yeast *Saccharomyces carlsbergensis*, named yeast ACBP types 1 and 2. Both proteins contain 86 amino acid residues and are identical except for four conservative substitutions. In comparison with human ACBP, yeast ACBPs exhibit 48 % (type 1) and 49 % (type 2) conservation of amino acid residues. The amino acid sequence of *S. carlsbergensis* ACBP type 1 was found to be identical with the one ACBP present in *Saccharomyces cerevisiae*. A recombinant form of this protein was expressed in *Escherichia coli* and *S. cerevisiae*, purified, and its acyl-CoA-binding properties were characterized by isoelectric focusing and microcalorimetric analyses. The yeast ACBP was found to bind acyl-CoA esters with high affinity (K_d 0.55×10^{-10} M). Overexpression of yeast ACBP in *S. cerevisiae* resulted in a significant expansion of the intracellular acyl-CoA pool. Finally, Southern-blotting analysis of the two genes encoding ACBP types 1 and 2 in *S. carlsbergensis* strongly indicated that this species is a hybrid between *S. cerevisiae* and *Saccharomyces monacensis*.

INTRODUCTION

Acyl-CoA-binding protein (ACBP) is a 10 kDa protein which has been isolated and characterized independently in five different laboratories on the basis of its ability to (1) displace diazepam from the benzodiazepine-recognition site of the γ-aminobutyric acid receptor (Guidotti et al., 1983; Shoyab et al., 1986), (2) induce synthesis of medium-chain acyl-CoA esters by goat mammary-gland fatty acid synthetase (Mogensen et al., 1987), (3) suppress glucose-induced insulin secretion from isolated islets and perfused pancreas of the rat (Chen et al., 1988) and (4) stimulate pregnenolone synthesis in bovine adrenal-gland mitochondria (Yanagibashi et al., 1988; Besman et al., 1989).

Convincing evidence for a function for ACBP in acyl-CoA metabolism has been obtained. It has been shown that it specifically binds medium- and long-chain acyl-CoA esters with high affinity, whereas it does not bind CoA, non-esterified fatty acids, acyl-carnitines and a number of nucleotides (Rosendal et al., 1993). ACBP has been shown to protect acetyl-CoA carboxylase and the mitochondrial ATP/ADP translocase against inhibition by long-chain acyl-CoA esters (Rasmussen et al., 1993). It also stimulated acyl-CoA synthesis by the mitochondrial acyl-CoA synthetase (Rasmussen et al., 1993). Expression of recombinant bovine ACBP in yeast led to an expansion of the pool of acyl-CoA esters that correlated with the level of ACBP expression (Mandrup et al., 1993a). Finally, determination by n.m.r. spectroscopy of the three-dimensional structure of recombinant bovine ACBP complexed with hexadecanoyl-CoA has clearly revealed the nature of the specific binding of acyl-CoA to ACBP (Kragelund et al., 1993).

If ACBP indeed is involved in general acyl-CoA metabolism, a protein homologous to ACBP would be expected to be ubiquitously expressed in eukaryotes. Indeed, ACBP has recently been identified in plants (J. Ohlrogge, personal communication) and insects (Snyder and Feyereisen, 1993) and a yeast gene encoding a polypeptide of 87 amino acids exhibiting a striking similarity to mammalian ACBP has recently been identified and characterized (Rose et al., 1992). In the present paper, we report on the isolation of two 10 kDa proteins from the BK 2208 *Saccharomyces carlsbergensis* lager strain and show by amino acid sequence and functional analysis that these proteins are true ACBP homologues.

EXPERIMENTAL

Materials

Yeast nitrogen base (without amino acids) was from Difco Laboratories. Q-Sepharose Fast Flow, Sephadex G-100 and Sephadex G-50 superfine were obtained from Pharmacia. Nucleosil ODS (10 μm particle size, 100 nm pore size and 10 μm particle size, 300 nm pore size) was from Macherey-Nagel, Düren, Germany, Superspher C18 was from Merck. *Staphylococcus aureus* V8 proteinase (EC 3.4.21.19) was from Miles Laboratories, and trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), restriction enzymes and other nucleic acid-modifying enzymes were from Boehringer, Mannheim, Germany. Propan-2-ol (h.p.l.c. grade) and trifluoroacetic acid (gas-phase sequenator grade) were from Rathburn, Walkerburn, Scotland, U.K. Oligonucleotides were obtained from DNA Technology, Aarhus, Denmark. *Taq* DNA polymerase was from Perkin–Elmer–Cetus, and Perfect Match DNA polymerase enhancer was obtained from Stratagene. Radionucleotides were from Du Pont–New England Nuclear.

Strains

The following *Saccharomyces cerevisiae* strains were used. Strain X2180-1A:(*SUC2 mal mel gal2 CUP1*) was obtained from sporulation of an a/α diploid that arose in strain S288C (Mortimer and Contopoulou, 1991). Strain Y700 (*Mat* α, *ade-1, his3, ura3, trp1, leu2*), a haploid strain of the diploid W303

Abbreviations used: ACBP, acyl-CoA-binding protein; 12-DSC, 12-doxylstearoyl-CoA.

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(Sorger and Pelham, 1988), was supplied by Dr. Bent Jacobsen, Institute of Molecular Biology, University of Aarhus, Denmark. The *Saccharomyces monacensis* strain was CBS 1503: bottom-fermenting strain no. II supplied by Dr. Mogens Bohl Pedersen, Carlsberg Laboratory, Copenhagen, Denmark. The *S. carlsbergensis* was the BK 2208 lager strain (Casey and Pedersen, 1988). *E. coli* strain DH5α (*F⁻, recA1, gyrA96, thi, hsdR17* (r_k^-, m_k^+), *sup E44, relA1,* λ-*φ80 dlac2* Δ*M15*) was used for propagation of plasmids and for heterologous expression of yeast ACBP.

Purification of ACBP from *S. carlsbergensis*

S. carlsbergensis cell suspension (approx. 300 ml of packed cells) was obtained from the Albani Brewery, Odense, Denmark. The pH was adjusted to 3.9 by addition of acetic acid, and the suspension was then passed through a French press twice at a pressure of 6210–8280 kPa. The pH was then adjusted to 7.0 with 5 M NaOH and the solution cleared by centrifugation at 10000 *g* for 20 min. ACBP in the supernatant was purified as described for bovine liver ACBP (Hach et al., 1990) except that ACBP in the eluate from the ion-exchange column was monitored by e.p.r. spectroscopy. Fractions from two well-resolved peaks that quenched the e.p.r. signal of 12-doxylstearoyl-CoA (12-DSC) were pooled separately and further purified by reversed-phase h.p.l.c. on a Nucleosil ODS 10/100 C18 column (8 mm × 120 mm) equilibrated with 20 % solution B [50 % (v/v) propan-2-ol, 0.1 % (v/v) trifluoroacetic acid in water] in solution A [0.1 % (v/v) trifluoroacetic acid in water]. Proteins were eluted with a gradient of solution B in solution A as follows: 20 % B to 80 % B for 42 min with a flow of 3 ml/min.

E.p.r. spectroscopy

12-DSC was synthesized and purified as described by Rasmussen et al. (1990). E.p.r. measurements were carried out as described by Rosendal et al. (1993).

Electrophoresis and isoelectric focusing

All electrophoretic and isoelectric focusing analyses were performed on a Pharmacia Phast System using either 20 % (w/v) acrylamide gels or isoelectric focusing gels with ampholytes for the pH range 3–9. Analyses were carried out as recommended by the manufacturer. For analysis of acyl-CoA-binding, ACBP was incubated with hexadecanoyl-CoA before isoelectric focusing.

Enzymic digestion of yeast ACBP

For tryptic and chymotryptic digestion, 2 nmol of yeast ACBP type 1 was dissolved in 100 μl of 100 mM NH_4HCO_3, pH 8.2, and 2 % (w/w, protein/protein) trypsin or chymotrypsin was added, and the digestion was allowed to take place at 37 °C for 2 h. For *Staph. aureus* protease digestion, 2 nmol of yeast ACBP type 1 was dissolved in 100 μl of 100 mM ammonium acetate, pH 4.0, and 5 % (w/w, protein/protein) *Staph. aureus* V8 protease was added, and the digestion was allowed to take place overnight at 37 °C. For endoproteinase ASP-N digestion, 2 nmol of yeast ABCP type 2 was dissolved in 100 μl of 100 mM NaH_2PO_4, pH 7.8, and 2 % (w/w, protein/protein) endoproteinase Asp-N was added, and the digestion was allowed to take place at 37 °C for 4 h. All digestions were terminated by injecting the reaction mixture directly on to the h.p.l.c. column. Separation of peptides in the enzymic digest was performed on a column (4 mm × 250 mm) packed with Nucleosil C18 (300 nm pore size, 10 μm particle size) using a gradient of buffer B (90 % acetonitrile, 10 % water) in buffer A (0.1 % trifluoroacetic acid in water).

Sequence analysis

Sequence analysis was performed on a Knauer 810 pulsed-liquid sequencer equipped with an on-line Applied Biosystems 140B/759A h.p.l.c. system. Chemicals were used as supplied by Knauer except that 60 μl of cyclohexamine was added to each bottle of S1 and S2 (1-chlorobutane and ethyl acetate respectively). Separation of phenylthiohydantoin amino acid residues was carried out on a home-packed 2 mm × 250 mm column (Knauer) packed with Superspher C18 (5 μm) using a gradient of acetonitrile in 12 mM sodium acetate, pH 3.7, containing 7 % acetonitrile and 0.01 % trimethylamine.

M.s.

The masses of intact yeast ACBP type 1 and 2 were determined by electrospray m.s. using a Vestec instrument (Vestec Corp., Houston, TX, U.S.A.). The purified proteins were dissolved in 1 % acetic acid/50 % methanol to a concentration of 0.2 μg/μl and introduced via a syringe pump with a flow rate of 0.3 μl/min. Spectra were acquired in the positive ion mode at 10 s/scan and a mass window of m/z 600–1700 using a Teknivent Vector 2 data system. Ten scans were averaged. The molecular mass of the protein was calculated by weighted averaging as described by Mann et al. (1989). The spectrometer was independently calibrated using the multiply charged envelope of recombinant bovine ACBP (Mandrup et al., 1991). The masses of all peptides derived from the two ACBP polypeptides were determined on a Bioion 20 K plasma-desorption time-of-flight mass spectrometer. The peptides were dissolved in 0.1 % trifluoroacetic acid, and 10–100 pmol was applied in 2–3 μl to an aluminized Mylar foil coated with nitrocellulose and spin-dried (Nielsen et al., 1988). The spectra were accumulated for 5×10^5–10^6 fission events at 15–16 kV acceleration voltage and calibrated on the basis of the H^+ and NO^+ ions.

DNA isolation and Southern blotting

DNA was isolated from the different yeast species by standard techniques using zymolase digestion (Ausubel et al., 1991). Digestion with the indicated restriction enzymes was performed as recommended by the manufacturer. Digested DNA was fractionated by electrophoresis through 0.7 % agarose gels and blotted on to Zetaprobe nylon membranes (Bio-Rad) using a PosiBlot pressure blotter (Stratagene). DNA was cross-linked to the membranes by u.v. irradiation using a Stratalinker u.v. crosslinker (Stratagene). Hybridization was performed by standard techniques (Sambrook et al., 1989) using moderately stringent conditions for hybridization and washing. The probe was a 467 bp *Spe*I–*Dra*I fragment covering the coding region of the *S. cerevisiae* ACBP gene plus 17 bp of the 5′-leader and 186 bp of the 3′-trailer. The probe was ^{32}P-labelled by the random-primer procedure (Feinberg and Vogelstein, 1983).

Construction of plasmids expressing *S. cerevisiae* ACBP

Two sets of primers were used for amplification of the *S. cerevisiae* ACBP gene. Primer set 1 consisted of pr3726, 5′-GCGGAATTCAAAATGGTTTCCCAATTATTCG-3′, corresponding to position −3 to +19 (+1 is the A in the ATG translation start codon) of the ACBP gene (Rose et al., 1992) with an extension containing an *Eco*RI recognition site, and

pr3972, 5′-GCGAAGCTTTCTAAGAGGAGTACTTGG-3′, corresponding to position +267 to +248 of the ACBP gene with an extension containing a *Hin*dIII recognition site. Primer set 2 consisted of pr3726 and pr3864, 5′-GTAAGTGTACTGTACCACTAG-3′, corresponding to position +754 to +734 of the ACBP gene.

Amplification of the *S. cerevisiae* gene with primer set 1 or 2 was performed as follows: 100 ng of *S. cerevisiae* strain X2180-1A genomic DNA was combined with 100 pmol of each primer in 50 μl of 20 mM Tris/HCl, pH 8.3, containing 100 mM KCl and 3 mM $MgCl_2$ and denatured at 95 °C for 5 min. Then 50 μl of dATP, dCTP, dGTP and dTTP (each at a concentration of 400 μM) containing 2.5 units of *Taq* DNA polymerase and 1 unit of Perfect Match was added, and amplification according to the following programme was initiated: denaturation for 1 min at 94 °C, annealing for 2 min at 50 °C, and extension for 1.5 min at 72 °C. A total of 30 cycles was performed. The PCR products were extracted once with chloroform, precipitated with ethanol, digested with *Eco*RI and *Hin*dIII (primer set 1) or *Eco*RI and *Spe*I (primer set 2) and then purified from a low-temperature melting agarose gel.

The *Eco*RI–*Hin*dIII fragment was ligated into the expression vector pKK223-3 (Pharmacia) digested with the same enzymes, and transformed into *E. coli* DH5α. This construct was named pKKyACBP. The *Eco*RI–*Spe*I fragment was ligated into a modified pYES2 vector (Invitrogen) digested with *Eco*RI and *Xba*I, and transformed into *E. coli* DH5α. This construct was named pTPIyACBP. In the modified pYES2 vector the *GAL1* upstream activating and promoter sequences had been replaced by a *Sph*I–*Eco*RI fragment containing the promoter of the *S. cerevisiae* triose phosphate isomerase, *TPI1*, gene. This modified vector was obtained from Dr. Nanni Din, Novo Nordisk, Bagsvaerd, Denmark.

The ACBP inserts in pKKyACBP and pTPIyACBP were sequenced by the dideoxynucleotide-chain-termination method (Sanger et al., 1977).

Production of recombinant *S. cerevisiae* ACBP in *E. coli* and *S. cerevisiae*

E. coli DH5α transformed with pKKyACBP was grown in a 5-litre fermenter with pH control, and ACBP was purified as described previously (Mandrup et al., 1991). *S. cerevisiae*, strain Y700 transformed with the pTPIyACBP, was also grown in a 5-litre fermenter with pH control, 5 litres of the medium contained 50 g of succinic acid, 30 g of NaOH, 10 g of $(NH_4)_2PO_4$, 8.75 g of KH_2PO_4, 5 g of $MgSO_4,7H_2O$, 1.25 g of K_2HPO_4, 1 g of NaCl, 4.8 g of threonine, 3.75 g of serine, 3 g of aspartic acid, 1 g of glutamic acid, 0.6 g of methionine, 0.5 g of phenylalanine, 0.4 g of alanine, 0.3 g of valine, tryptophan, tyrosine and lysine, 0.2 g of arginine and histidine hydrochloride, 200 g of glucose, 1.05 g of $CaCl_2$ and 10 ml of a vitamin stock solution containing 40 mg of thiamin, 40 mg of pyridoxine, 40 mg of pantothenate and 0.2 mg of biotin per 100 ml. Cells were harvested and ACBP was purified as described above (Mandrup et al., 1991).

Extraction and reversed-phase h.p.l.c. of acyl-CoA esters

Y700 transformed with pTPIyACBP was grown in 5-litre conical flasks with 500 ml of medium containing 20 g/l glucose, 8 g/l yeast nitrogen base, 60 mg/l isoleucine, leucine and phenylalanine, 55 mg/l adenine sulphate and tyrosine, 50 mg/l threonine, 40 mg/l lysine and tryptophan, 20 mg/l arginine and 10 mg/l histidine and methionine. Y700 as wild-type was grown in the same medium containing 55 mg/l uracil. The yeast cells (approx. 5×10^9) were harvested and used for analysis of acyl-CoA esters as described by Mandrup et al. (1993a).

Determination of dissociation constant of hexadecanoyl-CoA binding to ACBP by titration microcalorimetry

Calorimetric experiments were carried out using the OMEGA titration calorimeter (MicroCal, Northampton, MA, U.S.A.) with a 100 μl injection syringe and stirring at 400 rev./min. The concentration of hexadecanoyl-CoA in the syringe was approximately 20 times that of the ACBP concentration (0.02 mM) in the reaction cell. The reference cell of the calorimeter was filled with water. Protein and ligand were both in a 25 mM ammonium acetate buffer, pH 6.0.

Software supplied by the manufacturer (ORIGIN; Microcal) or Calreg (version 3.0) was used for integration of heat signals as well as non-linear regression analysis and plotting of the results.

Determination of ACBP content in yeast.

Determination of ACBP by e.l.i.s.a. was as described previously (Mandrup et al., 1993), except that rabbit anti-(yeast ACBP) and biotinylated rabbit anti-(yeast ACBP) antibodies were used.

RESULTS

Isolation of yeast ACBP homologues

The e.p.r. signals of unbound 12-DSC were found to be completely quenched on binding to ACBP (Rosendal et al., 1993). Therefore we decided to employ this property to monitor fractions during attempts to isolate ACBP-like proteins from the yeast *S. carlsbergensis*. We were able to locate two peaks in the eluate from a Q-Sepharose ion-exchange column that strongly quenched the e.p.r. signals of added 12-DSC. Further fractionation by reversed-phase h.p.l.c. resulted in the purification of two proteins named yeast ACBP type 1 and yeast ACBP type 2. By electrospray m.s. the M_r of yeast ACBP type 1 was determined to be 9929.7 ± 0.7 and that of yeast ACBP type 2 was 9952.1 ± 1.3. As expected, both purified proteins quenched the e.p.r. signal of added 12-DSC (results not shown).

The primary structure of yeast ACBP types 1 and 2

The primary structures of ACBP types 1 and 2 were determined by a combination of conventional Edman degradation and m.s. analysis (Roepstorff and Højrup, 1993). Unlike ACBP found in higher eukaryotes, that found in yeast was not N-terminally acetylated. Edman degradation of the intact yeast ACBP type 1 established the identity of the first 45 residues. The rest of the sequence was determined from peptides generated by digestion with trypsin, chymotrypsin or *Staph. aureus* protease as shown in Figure 1(a). The identity of the C-terminal residue (Ser-86) was not determined by protein sequencing, but the identity was established by the plasma-desorption spectrum of the C-terminal *Staph. aureus* protease peptide (results not shown) and the mass of the intact protein (9930.1). The precision of these results leaves no doubt about the identity of the terminal residue. The intact ACBP type 2 was sequenced for 68 cycles, and the rest of the sequence was determined from peptides resulting from digestion with endoproteinase Asp-N. No overlap was established for the two C-terminal peptides, but homology considerations as well as the mass of the intact protein showed the sequence in Figure 1(b) to be the only one possible.

Comparison of ACBP type 1 with ACBP type 2 shows that the two proteins are identical except for four conservative sub-

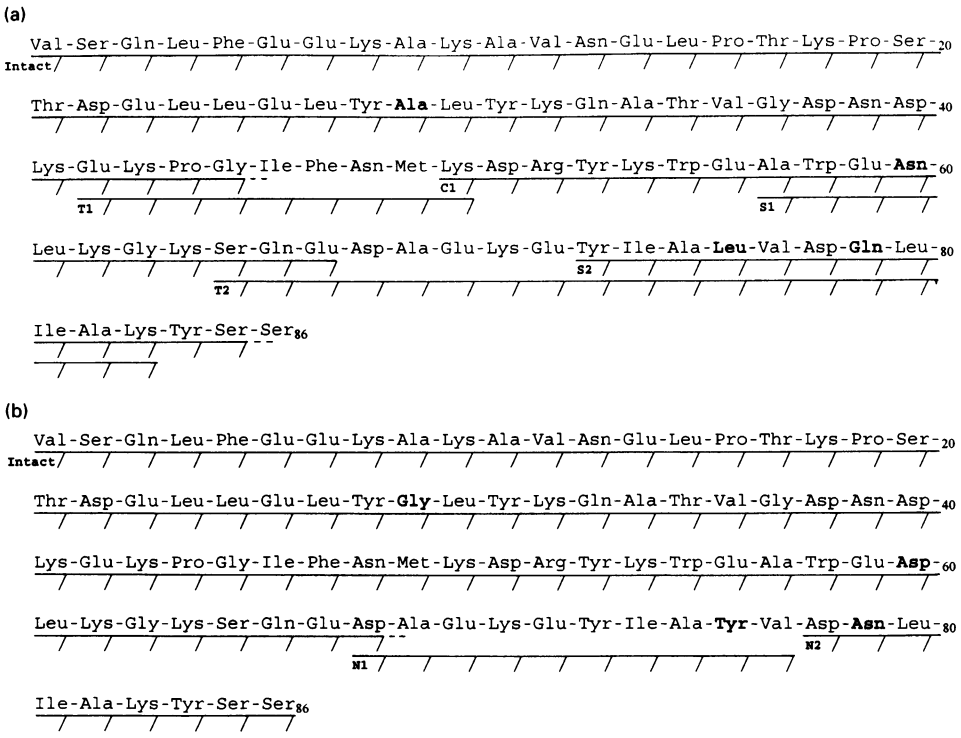

Figure 1 Complete primary structure of (a) yeast ACBP type 1 and (b) yeast ACBP type 2

Shown beneath each sequence are the peptides used to construct the sequence. / indicates residue identified by Edman sequencing. Residues that differ in the two sequences are shown in bold. Abbreviations: Intact, Edman sequencing carried out on the intact protein; T, tryptic peptide; C, chymotryptic peptide; S, *Staph. aureus* proteinase peptide; N, endoproteinase Asp-N peptide. See the Materials and methods section for experimental details.

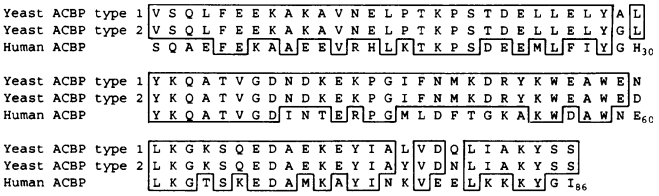

Figure 2 Alignment of yeast ACBP types 1 and 2 and human ACBP

Identical amino acid residues are boxed.

stitutions: position 29, Ala/Gly; position 60, Asn/Asp; position 76, Leu/Tyr; and position 79, Gln/Asn.

Yeast ACBP types 1 and 2 exhibit 48% and 49% conservation of amino acid residues respectively in comparison with human ACBP (Figure 2). It is noteworthy that those amino acid residues (Ala-9, Tyr-28, Lys-32, Lys-54, Tyr-73) that have been shown by n.m.r. spectroscopy to be important for binding of acyl-CoA esters (Kragelund et al., 1993) are fully conserved.

Functional analysis of yeast ACBP types 1 and 2

The conservation of the primary structures of yeast and mammalian ACBP indicates that yeast ACBP, like mammalian ACBP, binds acyl-CoA esters. However, considering the diversity of functions associated with mammalian ACBP, it was important to undertake a thorough functional characterization of the yeast protein. A convenient way of analysing binding of acyl-CoA to ACBP is isoelectric focusing. Binding of the negatively charged hexadecanoyl-CoA to mammalian ACBP results in a shift in isoelectric point of the complex compared with that of the free protein. However, the amount of protein isolated from *S. carlsbergensis* did not allow comprehensive binding studies. Therefore the expression vector pKKyACBP containing the coding region of the *S. cerevisiae* ACBP gene was constructed and *S. cerevisiae* ACBP was expressed in *E. coli*. Electrospray m.s. analysis of the purified protein expressed in *E. coli* revealed that only 45% of the ACBP was correctly processed (M_r 9932). The remaining 55% still contained the initiating methionine (M_r 10063). To circumvent this problem, the yeast expression vector pTPIyACBP was constructed. In Y700 cells transformed with pTPIyACBP, ACBP was found to constitute 3–4% of total protein. ACBP purified from Y700/pTPIyACBP cells contained less than 12% of unprocessed ACBP, as determined by electrospray m.s., and strongly quenched the e.p.r. signal of 12-DSC (results not shown).

Binding of hexadecanoyl-CoA was tested by isoelectric focusing (Figure 3). Whereas hexadecanoyl-CoA when present in threefold molar excess over ACBP completely shifted recombinant bovine ACBP from focusing at pH 6.5 to pH 4.4 (lanes 1 and 2), the pI of the recombinant *S. cerevisiae* ACBP expressed in either *E. coli* (lane 3) or *S. cerevisiae* (lane 6) was only partly shifted by a threefold excess of the ligand. An increase in the ligand concentration to 10× and 30× the ACBP concentration used (lanes 5 and 6) did not change this pattern. A binding test with purified native ACBP from *S. carlsbergensis* yielded results similar to those obtained with recombinant ACBP (results not shown). Electrospray m.s. of ACBP purified from either *E. coli*

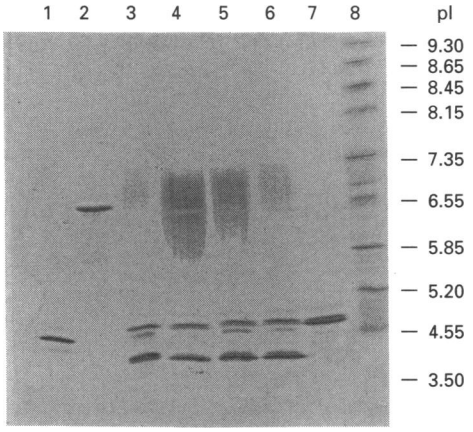

Figure 3 Isoelectrofocusing of recombinant bovine ACBP and recombinant *S. cerevisiae* ACBP in the absence or presence of hexadecanoyl-CoA

Lane 1, bovine ACBP plus 3-fold molar excess of acyl-CoA; lane 2, bovine ACBP; lane 3, *S. cerevisiae* ACBP expressed in *E. coli* plus 3-fold molar excess of acyl-CoA; lanes 4–7, *S. cerevisiae* ACBP expressed in *S. cerevisiae* with 30-fold (lane 4), 10-fold (lane 5) and 3-fold (lane 6) molar excess of acyl-CoA and without acyl-CoA (lane 7); lane 8, pI markers.

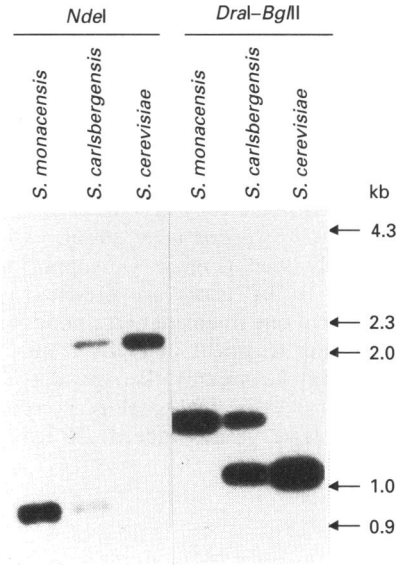

Figure 4 Southern blotting of DNA from *S. cerevisiae*, *S. monacensis* and *S. carlsbergensis*

DNA was digested with the indicated restriction enzymes, and the fragments were separated in 0.7% agarose gels and blotted on to a nylon membrane. The blot was hydridized with a probe covering the coding region of the *S. cerevisiae* ACBP gene.

or *S. cerevisiae* showed that it contained ACBP only either with or without the initiating methionine (results not shown). The isoelectric points of both recombinant proteins were shifted to the same extent on incubation with hexadecanoyl-CoA, even though the ACBP expressed in *E. coli* contained 55% unprocessed ACBP. These results therefore suggest that the incomplete shift cannot be caused by the inability of the unprocessed ACBP to bind acyl-CoA. The isoelectric point of *S. cerevisiae* ACBP is 4.7 (4.81 calculated from the amino acid sequence) and the ACBP–hexadecanoyl-CoA complex focuses at a pH of about 4.1. A possible explanation for the incomplete binding is therefore that the protein was partially unfolded when shifted to pH 4.1. Alternatively the incomplete shift might be due to the fact that yeast ACBP has a lower binding affinity for acyl-CoA than has bovine ACBP. Two separate experiments to determine the K_d for hexadecanoyl-CoA binding to *S. cerevisiae* ACBP by titrating *S. cerevisiae* ACBP with hexadecanoyl-CoA in a titration microcalorimeter gave values of 0.85×10^{-10} M and 0.25×10^{-10} M. The K_d for hexadecanoyl-CoA binding to bovine ACBP has been determined to be 0.45×10^{-13} M (Rasmussen et al., 1994). However, the calorimetry binding results, together with those obtained by isoelectric focusing, show that yeast ACBP binds acyl-CoA esters with high affinity. This conclusion is further supported by experiments in which yeast ACBP was overexpressed in *S. cerevisiae*. We have previously shown that expression of bovine ACBP in *S. cerevisiae* resulted in a dose-dependent increase in the total pool of acyl-CoA esters accompanied by an increase in the relative contribution of the $C_{16:0}$ acyl-CoA ester to the total pool of acyl-CoA (Mandrup et al., 1993a). Table 1 shows that similar increases in the acyl-CoA pool were observed in Y700 transformed with pTPIyACBP.

Genome organization of the ACBP genes in *S. carlsbergensis*

The isolation of two forms of the ACBP protein from the BK 2208 *S. carlsbergensis* lager strain immediately suggested that two functional ACBP genes are present in this strain. On the basis of hybridization data, it was previously suggested that the *S. carlsbergensis*-type strain and the lager strains are sibling hybrid species produced by hybridization of an *S. cerevisiae* top-fermenting strain with a bottom-fermenting strain, *S. monacensis* (Pedersen, 1986). While the present work was in progress, molecular cloning of the *S. cerevisiae* ACBP gene was reported (Rose et al., 1992). Interestingly, the predicted amino acid sequence of the *S. cerevisiae* ACBP protein is identical with that of the *S. carlsbergensis* type-1 ACBP protein. To investigate

Table 1 Content of dominant acyl-CoA esters in *S. cerevisiae* strain Y700 and Y700 transformed with the expression vector pTPIyACBP

Values for acyl-CoA concentrations are means of double determinations ± half the difference; 5×10^9 cells correspond to 500 A_{550} units. For experimental details, see the Materials and method section.

	Acyl-CoA (nmol/5×10^9 cells)						ACBP (nmol/5×10^9 cells)
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	Total	
Y700	0.14 ± 0.01	0.42 ± 0.01	2.30 ± 0.32	0.13 ± 0.02	0.38 ± 0.11	3.37 ± 0.38	0.7 ± 0.0
Y700/ pTPIyACBP	0.69 ± 0.22	1.71 ± 0.38	3.23 ± 0.73	0.24 ± 0.05	0.67 ± 0.11	6.54 ± 1.49	27.7 ± 4.5

further the genomic organization of the ACBP genes in the BK 2208 *S. carlsbergensis* lager strain, DNA from this strain, *S. cerevisiae* and *S. monacensis* were analysed by Southern blotting using the 458 bp *SpaI–DraI* fragment of the *S. cerevisiae* ACBP gene as a probe. Figure 4 shows that only one fragment in the different digests of *S. cerevisiae* DNA and *S. monacensis* DNA hybridized to the *S. cerevisiae* ACBP gene probe, indicating that ACBP is encoded by a single-copy gene in these two species. In DNA from the *S. carlsbergensis* lager strain, two hybridizing fragments are clearly seen (Figure 4), supporting the view that two functional ACBP genes are present in this strain. Interestingly, the size of one fragment corresponds exactly to the size of the hybridizing fragment in DNA from *S. cerevisiae*, whereas the other matches exactly the size of the hybridizing fragment in *S. monacensis* DNA, thus corroborating the hypothesis that the lager strains indeed are hybrids between *S. cerevisiae* and *S. monacensis*.

DISCUSSION

The present results clearly demonstrate that a true ACBP homologue is present in the lower eukaryote, yeast. Analysis of the primary structure of yeast ACBP strongly suggests that its secondary and tertiary structures are similar to those of vertebrate ACBP. In keeping with this, yeast ACBP, like vertebrate ACBP, is able to bind acyl-CoA esters with high affinity, as determined by microcalorimetry and isoelectric focusing. We have shown that expression of bovine ACBP in yeast results in an increase in the pool size of acyl-CoA esters (Mandrup et al., 1993a). The results in Table 1 show that the pool size of acyl-CoA in yeast also responds to changes in the intracellular level of yeast ACBP.

The mere presence of a true ACBP homologue in yeast, plants and insects has an important conceptual implication for our view on the possible biological function(s) of ACBP. Given the fact that binding of acyl-CoA esters is the only unambiguously demonstrated property of ACBP (Knudsen and Nielsen, 1990), the ubiquitous presence of ACBP is compelling support for the view that the function of ACBP is related to acyl-CoA metabolism and function. A more general function of ACBP common to all types of cells and related to its ability to bind, protect and transport acyl-CoA esters, as demonstrated by Rasmussen et al. (1993, 1994), is supported by the fact that the ACBP gene contains all the hallmarks of a typical house-keeping gene (Mandrup et al., 1992, 1993b).

Apart from a role in lipid metabolism, acyl-CoA esters are indispensable for protein acylation. Numerous proteins involved in signal transduction are subject to dynamic acylation–deacylation reactions that profoundly influence their biological activity [for reviews see Schultz et al. (1988), Deschenes et al. (1990) and Casey (1992)]. The mechanism by which *N*-myristoylation proceeds predicts that the availability of myristoyl-CoA is important for the regulation of this process (Rocque et al., 1993). Furthermore, it has been suggested that some of the differences in the nature of the attached fatty-acyl groups may be determined by the relative abundance of the different acyl-CoA esters in the cells (Kokame et al., 1992). These are intriguing suggestions in relation to our observation that expression of bovine recombinant ACBP in yeast (Mandrup et al., 1993a) as well as overexpression of yeast ABCP not only expanded the pool size of acyl-CoA but also significantly increased the relative abundance of $C_{16:0}$ acyl-CoA and decreased the relative abundance of $C_{18:0}$ and $C_{18:1}$ acyl-CoA.

Acyl-CoA esters have been implicated in signal transduction by interacting with the thyroid hormone receptor (Li et al., 1990, 1993) and protein kinase C (Bronfman et al., 1988), and recent experiments have clearly demonstrated that, by binding to transcription factors, acyl-CoA esters are able to regulate transcription of genes involved in lipid metabolism (Henry and Cronan, 1992; DiRusso et al., 1992). Furthermore, acyl-CoA esters are required for budding off and fusion of transport vesicles in secretory pathways in eukaryotes (Rothman and Orci, 1992).

All these diverse functions involving Acyl-CoA esters require a system for their transport and delivery. We have shown that ACBP can fulfil such a role (Rasmussen et al., 1994). Thus the identification of a true ACBP homologue in yeast that is amenable to genetic analysis opens up possibilities for detailed studies of the biological function of this ubiquitous protein.

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