Acyl-CoA-binding protein (ACBP) can mediate intermembrane acyl-CoA transport and donate acyl-CoA for β -oxidation and glycerolipid synthesis

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The dissociation constants for octanoyl-CoA, dodecanoyl-CoA and hexadecanoyl-CoA binding to acyl-CoA-binding protein (ACBP) were determined by using titration microcalorimetry. The K_D values obtained, $(0.24 \pm 0.02) \times 10^{-6}$ M, $(0.65 \pm 0.2) \times 10^{-8}$ M and $(0.45 \pm 0.2) \times 10^{-13}$ M respectively, were much lower than expected. ACBP was able to extract hexadecanoyl-CoA

from phosphatidylcholine membranes immobilized on a nitrocellulose membrane. The acyl-CoA/ACBP complex formed was able to transport acyl-CoA to mitochondria or microsomes in suspension, or to microsomes immobilized on a nitrocellulose membrane, and to donate them to β -oxidation or glycerolipid synthesis in mitochondria or microsomes, respectively.

INTRODUCTION

Acyl-CoA-binding protein (ACBP) is a highly conserved cytosolic protein, consisting of 86 amino acid residues with an acetylated N-terminus (Mikkelsen et al., 1987; Knudsen et al., 1989). ACBP was found to bind acyl-CoA esters of chain length C_{14} – C_{22} with high affinity (Mikkelsen and Knudsen, 1987; Rosendal et al., 1993). By using photoreactive acyl-CoA esters, the hydrophobic binding site on bovine ACBP was determined to include amino acids 23–28 (Hach et al., 1990), which was assigned to the second of the four α -helices of ACBP (Andersen et al., 1991).

ACBP has also been suggested to be a modulator of neuroactivity, and to be involved in regulation of steroid-hormone synthesis and insulin secretion (for a review see Knudsen et al., 1993). However, conclusive evidence for the involvement of ACBP in these functions is still lacking (Knudsen and Nielsen, 1990).

That ACBP participates in acyl-CoA metabolism is supported by its ability to bind with high affinity and to sequester long-chain acyl-CoA esters (Mikkelsen and Knudsen, 1987; Rasmussen et al., 1990; Rosendal et al., 1993). These observations, and the fact that the genomic ACBP gene has all the characteristics of a housekeeping gene (Mandrup et al., 1992), indicate that ACBP is a housekeeping protein involved in acyl-CoA metabolism, and is not a protein with specific functions confined to specialized cells. The observation that the pool of long-chain acyl-CoA esters is dramatically increased when ACBP is over-expressed in yeast shows that ACBP also binds *in vivo* and thereby forms a pool of long-chain acyl-CoA esters (Mandrup et al., 1993).

Activation of long-chain fatty acids catalysed by the long-chain acyl-CoA synthetase (EC 6.2.1.3) is the first reaction in fatty acid metabolism. In spite of the central role of this enzyme in fatty acid metabolism, little is known about its regulation. However, long-chain acyl-CoA synthesis was shown to be feedback-regulated by hexadecanoyl-CoA with an apparent K_i of 4 μ M (Pande, 1973).

A large number of cellular processes have been reported to be affected by long-chain acyl-CoA esters (see, e.g., Wise and Kuo, 1983; Powell et al., 1985; Pfanner et al., 1989; Prentki et al., 1992). Two proteins, in particular, have been shown to be inhibited by extremely low concentrations of long-chain acyl-CoA esters, namely acetyl-CoA carboxylase (ACC; $K_i = 1$ –5 nM; Nikawa et al., 1979) and adenine nucleotide translocase (ANT; $K_i < 1$ μ M; Woldegiorgis et al., 1982).

We have recently shown that ACBP can effectively release product inhibition of long-chain acyl-CoA ester and protect ACC and ANT against inhibition by long-chain acyl-CoA at acyl-CoA/ACBP ratios ≤ 0.9 (Rasmussen et al., 1993). ACBP binds acyl-CoA esters with a C_8 – C_{22} acyl chain (Mikkelsen and Knudsen, 1987), and the relative binding affinity is highest for C_{14} – C_{22} acyl-CoA esters (Rosendal et al., 1993). In addition, we determined the apparent K_D values for hexadecanoyl-CoA binding to rat and bovine ACBP to be 0.27 μ M and 0.22 μ M respectively, using the Lipidex 1000 binding assay (Rasmussen et al., 1990). The Lipidex 1000 binding assay, however, does not give true K_D values for acyl-CoA binding. Rather, it reflects competitive binding between ACBP and Lipidex 1000 (Rosendal et al., 1993). The fact that less than 0.6% of the long-chain acyl-CoA synthesized by intact mitochondria in the presence of physiological concentration of ACBP remains bound to the mitochondria confirms the high binding affinity of ACBP for long-chain acyl-CoA esters (Rasmussen et al., 1993).

This observation also raises the question as to whether ACBP, in spite of the high binding affinity, can donate acyl-CoA esters back to mitochondria and other organelles, and whether ACBP can mediate long-chain acyl-CoA transport from one organelle to another.

The present paper reports determination of true K_D values for octanoyl-CoA, dodecanoyl-CoA and hexadecanoyl-CoA binding to ACBP by using titration microcalorimetry, and presents evidence which clearly demonstrates that ACBP can mediate transport of long-chain acyl-CoA esters from one membrane and donate them to an acyl-CoA-utilizing enzyme in a distinct membrane system.

Abbreviations used: ACBP, acyl-CoA-binding protein; ACC, acetyl-CoA carboxylase; ANT, adenine nucleotide translocase; CPT, carnitine palmitoyltransferase; FABP, fatty acid binding protein; MLV, multilamellar liposomal vesicle; MOM, mitochondrial outer membrane; PCS, phospholipid-coated sheet.

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MATERIALS AND METHODS

Materials

Sprague-Dawley male rats (200 g) and lactating rabbits (2–3 weeks *post partum*) were used. [$1\text{-}^{14}\text{C}$]Hexadecanoic acid, 1,2-hexadecanoyl-L-phosphatidyl[$N\text{-methyl-}^3\text{H}$]choline and [9,10- ^3H]hexadecanoic acid were from Amersham International, Amersham, Bucks., U.K. BSA and L-glycerol 3-phosphate were supplied by Boehringer, Mannheim, Germany. High-performance-t.l.c. silica-gel 60 plates were from Merck, Darmstadt, Germany. CoA and Percoll were from Pharmacia, Uppsala, Sweden. Nitrocellulose paper was supplied by Millipore, Bedford, MA, U.S.A. AG 1-X8 anion-exchange resin (200–400 mesh) was from Bio-Rad, Richmond, CA, U.S.A. All other chemicals were of analytical grade, supplied by either Merck or Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of recombinant bovine ACBP

Recombinant bovine ACBP was purified from *Escherichia coli* harbouring the ACBP expression plasmid pKK 223ACBP Δ EI as previously described (Mandrup et al., 1991).

Preparation of phospholipid-coated nitrocellulose sheets (PCSs)

These were prepared by adhering multilamellar liposomal vesicles (MLVs) to nitrocellulose membranes. MLVs were prepared as described by Rasmussen et al. (1990).

As a standard procedure, each nitrocellulose membrane (1 cm $\times$ 1 cm) was incubated separately for 2 h at 25 °C with MLVs (2 mM phosphatidylcholine and 0.65 mM cholesterol) in 1 ml of 350 mM Tris/HCl, pH 7.2 (buffer A), and finally washed twice in buffer A. This procedure resulted in binding of 70 nmol of phosphatidylcholine/cm² of nitrocellulose membrane as determined by using 1,2-hexadecanoyl-L-phosphatidyl[$N\text{-methyl-}^3\text{H}$]choline for preparation of MLVs and subsequently PCSs. The PCSs carrying labelled MLVs were dissolved in 0.2 ml of acetone, and the radioactivity was determined by liquid-scintillation counting.

Loading of PCSs with acyl-CoA and desorption of acyl-CoA by ACBP

The PCSs were loaded with acyl-CoA by incubating with the indicated amount of either [$1\text{-}^{14}\text{C}$]hexadecanoyl-CoA (sp. radioactivity 12.04 $\mu\text{Ci}/\mu\text{mol}$) or [9,10- ^3H]hexadecanoyl-CoA (sp. radioactivity 0.82 mCi/ μmol) for 30 min at 25 °C, and washed twice in buffer A.

The ability of ACBP to desorb the acyl-CoA esters from the membranes was tested by incubating the indicated amount of ACBP with PCS loaded with [$1\text{-}^{14}\text{C}$]hexadecanoyl-CoA (1.3 nmol) for 30 min at 25 °C in buffer A (1 ml). At the end of the incubation, the acyl-CoA-loaded PCSs were removed and the amount of acyl-CoA bound to ACBP was determined by measuring the radioactivity in the medium by liquid-scintillation counting.

β -Oxidation assay

The carnitine-dependent oxidation of [9,10- ^3H]hexadecanoyl-CoA was measured by the formation of $^3\text{H}_2\text{O}$, essentially by the method of Moon and Rhead (1987). A PCS loaded with [9,10- ^3H]hexadecanoyl-CoA (0.2 nmol) was preincubated with mito-

chondria (0.1 mg of protein/ml) with or without binding protein for 5 min at 37 °C. The reaction was initiated by addition of L-carnitine (0.5 mM), and the incubation was allowed to proceed for the indicated time at 37 °C. The final volume was 1 ml. The reaction was stopped with 1 ml of 10% (w/v) trichloroacetic acid. Each sample was then centrifuged at 18 000 *g* for 5 min, the supernatant was transferred to another tube, and the pH raised to 8.5 by addition of NaOH. The supernatant was passed through a Dowex anion-exchange column (1 ml) equilibrated with water. The column was washed with a further 2 ml of water, and the amount of radioactivity in the total eluate was determined by liquid-scintillation counting.

Transport of acyl-CoA to microsomal phosphatidate synthesis

In a total volume of 1 ml, microsomes (0.1 mg of protein/ml) were preincubated with 2 mM L-glycerol 3-phosphate with or without ACBP for 5 min at 37 °C in 0.1 M potassium phosphate, pH 7.4 (buffer B). One PCS loaded with 1.5 nmol of [9,10- ^3H]hexadecanoyl-CoA was then added to each sample and incubated for the indicated time at 37 °C. At the end of the incubation, a portion (0.1 ml) of the incubation medium was mixed with chloroform/methanol (1:1, v/v; 0.65 ml). The amount of radioactivity and the lipid composition were then determined as described by Rasmussen et al. (1993).

Microsomes were fixed on nitrocellulose membranes (each 1 cm $\times$ 1 cm) by incubating the membranes with microsomal membranes (1 mg of protein/ml) for 18 h in buffer B at 25 °C with gentle mixing during the incubation period. PCS loaded with 1.6 nmol of [9,10- ^3H]hexadecanoyl-CoA (M1) and the nitrocellulose membranes with fixed microsomes (M2) were washed twice separately in buffer B and then incubated with or without ACBP in the presence of 2 mM L-glycerol 3-phosphate for the indicated time in buffer B at 37 °C. At the end of the incubation, M2 was removed and the lipid was extracted as described by Bligh and Dyer (1959). The efficiency of the extraction was increased by homogenizing the membranes with an Ultra-Turrax instrument before phase separation. The amount of radioactivity and the lipid composition were determined as described by Rasmussen et al. (1993).

Determination of dissociation constants of acyl-CoA binding to ACBP by titration microcalorimetry

All calorimetric experiments were carried out with 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 acyl-CoA in the syringe was approx. 20 times higher than 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 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 for non-linear regression analysis and plotting of the results.

RESULTS AND DISCUSSION

Knowing the true K_D for acyl-CoA binding to ACBP is necessary in order to calculate the free acyl-CoA concentration in kinetic studies with acyl-CoA-utilizing enzymes by using the acyl-CoA/ACBP complex as an acyl-CoA donor. In the present work we have determined K_D values for octanoyl-CoA, dodecanoyl-

Table 1 Determination of K_D values for octanoyl-CoA, dodecanoyl-CoA and hexadecanoyl-CoA binding to recombinant bovine ACBP

For experimental details see the Materials and methods section.

Acyl-CoA	K_D
Octanoyl-CoA	$(0.24 \pm 0.02) \times 10^{-6}$ M
Dodecanoyl-CoA	$(0.65 \pm 0.2) \times 10^{-8}$ M
Hexadecanoyl-CoA	$(0.45 \pm 0.2) \times 10^{-13}$ M

Figure 1 Microcalorimetric titration of recombinant bovine ACBP with octanoyl-CoA

ACBP dissolved in 25 mM ammonium acetate, pH 5.3, to a concentration of 0.02 mM was titrated with portions of 0.5 mM octanoyl-CoA, in the same buffer. For experimental details see the Materials and methods section; 1 cal $\approx$ 4.185 J.

CoA and hexadecanoyl-CoA binding to recombinant bovine ACBP by using titration microcalorimetry (Table 1). The titration curve for titration of ACBP with octanoyl-CoA is shown in Figure 1. The binding affinity of ACBP for all three acyl-CoA esters was much higher than expected from previous binding studies. The determined K_D of $(0.65 \pm 0.2) \times 10^{-8}$ M for dodecanoyl-CoA is two orders of magnitude lower than the apparent K_D for hexadecanoyl-CoA binding to ACBP determined previously by the Lipidex 1000 binding assay (Rasmussen et al., 1990).

The K_D for hexadecanoyl-CoA binding to ACBP could not be determined directly by microcalorimetric titration, due to its very high binding affinity. Therefore the dissociation constant for hexadecanoyl-CoA binding was obtained indirectly by titrating the ACBP/dodecanoyl-CoA complex with hexadecanoyl-CoA. The K_D obtained for hexadecanoyl-CoA binding to ACBP of $(0.45 \pm 0.2) \times 10^{-13}$ M (Table 1) is 4–6 orders of magnitude lower than the K_D reported for fatty acid binding to fatty-acid-binding protein (FABP) (Bass et al., 1993).

The very low K_D for acyl-CoA binding to ACBP is very important in relation to the possible regulatory role of acyl-CoA esters. It has recently been shown that the rat liver β_1 -thyroid receptor binds octadecanoyl-CoA with a K_D value of

0.12×10^{-6} M. The binding was specific and inhibited thyroid hormone binding (Li et al., 1993). The authors suggested that acyl-CoA esters might also bind to the thyroid receptor *in vivo* and therefore play a regulatory role in modifying the thyroid hormone signal; their major argument was that the β_1 -thyroid receptor binds acyl-CoA with about the same affinity as ACBP. However, this comparison was made with the previously published apparent K_D of 1.3×10^{-7} M (Rasmussen et al., 1990). With a cellular acyl-CoA/ACBP ratio of about 0.8 (see below) and a K_D for octadecanoyl-CoA binding to ACBP in the range of 10^{-13} M, the suggested role of acyl-CoA in regulating the thyroid receptor and many other cellular functions would have to be reconsidered. The key question is: what is the true K_D for acyl-CoA binding to the proteins suggested to be regulated by acyl-CoA? A major problem here is that acyl-CoA esters are strong detergents. They tend to adhere strongly to, e.g., test-tube walls, membranes, proteins, so that the true concentration is seldom known, and thus there is a need for standardization of techniques used for studying acyl-CoA binding. Titration microcalorimetry would appear to be one possibility.

The very high binding affinity of ACBP for long-chain acyl-CoA ester is in accordance with previously published data which show that ACBP competes with denatured membranes for acyl-CoA binding (Rasmussen et al., 1990), and protects ACC and the mitochondrial ANT against acyl-CoA inhibition (Rasmussen et al., 1993). In these studies it was also shown that ACBP can relieve mitochondrial acyl-CoA synthetase product inhibition and almost completely extract newly synthesized hexadecanoyl-CoA from mitochondrial membranes when present in large excess in the acyl-CoA synthetase assay.

The cellular concentration of ACBP and long-chain acyl-CoA in fed rats is about equimolar, and about 20 % of the acyl-CoA is located in the mitochondria (Rasmussen et al., 1993). From the analysis of more than 100 rats we found that the concentration of ACBP normally varied from 25 to 35 nmol/g of tissue (J. Knudsen, unpublished work). Occasionally, concentrations up to 50 nmol/g of tissue were found (Rasmussen et al., 1993). If it is assumed that the liver ACBP concentration is 30 μ M, the cytosolic acyl-CoA/ACBP ratio 0.8 and the average K_D for long-chain acyl-CoA binding to ACBP is 0.45×10^{-13} M, then the free cytosolic acyl-CoA concentration will be 0.2 pM.

The very high binding affinity and low free acyl-CoA concentration immediately raise the question as to which pathways ACBP can donate acyl-CoA ester. Can ACBP donate acyl-CoA esters once desorbed from the mitochondrial acyl-CoA synthetase back to mitochondria for β -oxidation? Furthermore, can ACBP extract acyl-CoA esters from one membrane and donate them to an acyl-CoA-consuming enzyme in a different membrane?

In order to answer these questions, a test system was designed in which ACBP is to extract acyl-CoA esters dissolved in multilamellar phosphatidylcholine liposomes immobilized on nitrocellulose sheets and deliver them to the carnitine palmitoyl-transferase (CPT) on intact mitochondria and thereby initiate β -oxidation. Figure 2(a) shows that increasing amounts of [1 - 14 C]hexadecanoyl-CoA were loaded on to PCSs when the concentration of acyl-CoA in the incubation mixture was increased. Within the range of concentrations used for these experiments, the percentage of acyl-CoA bound remained almost constant around 30 %. The amount of [1 - 14 C]hexadecanoyl-CoA extracted from PCSs loaded with hexadecanoyl-CoA increased linearly as a function of the amount of added ACBP (Figure 2b). The molar ratio of desorbed acyl-CoA to added ACBP was about 0.3.

The β -oxidation rate of [$9,10^3$ -H]hexadecanoyl-CoA immobilized on PCS incubated with mitochondria in the presence or

Figure 2 Loading of PCS with hexadecanoyl-CoA and desorption of hexadecanoyl-CoA by ACBP

The results are representative of individual experiments and are plotted as means $\pm$ range of duplicates. (a) PCSs (each 1 cm $\times$ 1 cm) were incubated with increasing amounts of [$1\text{-}^{14}\text{C}$]hexadecanoyl-CoA. The amount of acyl-CoA bound to the membranes was measured (□), and the percentage of added acyl-CoA bound to the membrane was calculated (▲). (b) PCSs loaded with 1.3 nmol of [$1\text{-}^{14}\text{C}$]hexadecanoyl-CoA were incubated with increasing amounts of ACBP, and the amount of desorbed acyl-CoA was measured. For further details see the Materials and methods section.

absence of 2.5 μM ACBP, BSA, or bovine liver FABP (L-FABP) is shown in Figure 3. The β -oxidation rate in the absence of any binding protein is very low. Addition of ACBP to the incubation medium increased the β -oxidation rate about 8-fold, whereas BSA and L-FABP only increased the rate 2.0- and 2.9-fold respectively, showing that ACBP is much more efficient in mediating hexadecanoyl-CoA transport than is BSA or L-FABP.

The molar ratio of hexadecanoyl-CoA to ACBP in solution in the experiment in Figure 4 would be 0.08, if all the hexadecanoyl-CoA was desorbed. Calculated from $K_D = 0.45 \times 10^{-13}$ M, the free hexadecanoyl-CoA concentration would be 2.3 fM. This shows that the driving force of the β -oxidation pathway is sufficiently high to compete with ACBP for very low acyl-CoA concentration, or that ACBP donates acyl-CoA directly to the mitochondria. Similar experiments were carried out with microsomes, where incorporation of [$1\text{-}^{14}\text{C}$]hexadecanoyl-CoA into phosphatidic acid was measured as a function of time with and without different concentrations of added ACBP (Figure 4). These results clearly demonstrate that ACBP is able to function also as a donor of acyl-CoA ester to microsomal acyltransferases. The present results, together with previously published data which show that ACBP can extract acyl-CoAs synthesized by mitochondria (Rasmussen et al., 1993), strongly indicate that

Figure 3 Effects of ACBP, L-FABP and BSA on the transport of immobilized acyl-CoA esters to carnitine-dependent β -oxidation in rat liver mitochondria

PCSs loaded with 0.2 nmol of [$9,10\text{-}^3\text{H}$]hexadecanoyl-CoA were prepared and incubated for the indicated time without (■) or with 2.5 μM of ACBP (△), L-FABP (◇) or BSA (▼) as described in the Materials and methods section. The results are representative of individual experiments and are given as means $\pm$ range of duplicates.

Figure 4 Effect of ACBP on the transport of immobilized long-chain acyl-CoA esters to phosphatidate synthesis in rabbit mammary-gland microsomal membranes

Microsomal membranes were incubated with PCS loaded with [$9,10\text{-}^3\text{H}$]hexadecanoyl-CoA (1.5 nmol) with (△, 5 μM ; ▼, 10 μM) or without (■) ACBP. For further details see the Materials and methods section. The results are representative of individual experiments and are shown as means $\pm$ range of duplicates.

acyl-CoA esters synthesized by mitochondria can be made available for microsomal glycerolipid synthesis, and vice versa.

The ultimate test for the acyl-CoA-transport ability of ACBP and its ability to donate acyl-CoA for glycerolipid synthesis would be to show transfer of acyl-CoA immobilized on one membrane to utilizing enzymes immobilized on a different membrane. The results in Figure 5 show that ACBP can indeed transport and donate acyl-CoA for glycerolipid synthesis in a time- and concentration-dependent manner in such a system.

In all the above experiments, the addition of ACBP to concentrations exceeding those which, from the results in Figure 2(b), must be assumed to extract all PCS-bound acyl-CoA, did not affect utilization of hexadecanoyl-CoA by the utilizing enzymes.

This phenomenon is clearly demonstrated in an experiment where mitochondrial β -oxidation of [$9,10\text{-}^3\text{H}$]hexadecanoyl-CoA immobilized on PCS in the presence of increasing concentrations of ACBP was measured (Table 2). At 10 μM ACBP maximal β -

Figure 5 Transport of long-chain acyl-CoA esters from PCSs to phosphatide synthesis by immobilized rabbit mammary-gland microsomal membranes

PCSs loaded with [9,10-³H]hexadecanoyl-CoA (1.6 nmol) were incubated with microsomes immobilized on nitrocellulose membranes in the absence (■) or presence of ACBP (▼, 5 μM; ◇, 10 μM; △, 20 μM). For further details see the Materials and methods section. The results are representative of individual experiments and are given as means ± range of duplicates.

Table 2 Carnitine-dependent β-oxidation of [9,10-³H]hexadecanoyl-CoA (0.2 nmol initially) immobilized on PCS in the presence of increasing amounts of ACBP

The samples were incubated for 30 min and analysed as described in the Materials and methods section. Results are given as means ± S.D. of triplicates.

ACBP (μM)	[9,10- ³ H]Hexadecanoyl-CoA oxidized (pmol)
—	4.42 ± 2.39
0.5	5.17 ± 0.75
2.5	12.05 ± 0.15
10	16.78 ± 1.32
20	15.48 ± 0.88

oxidation was obtained. At this concentration of ACBP it can be assumed that all PCS-bound acyl-CoA is desorbed (see Figure 2b). It is surprising that doubling the amount of ACBP from 10 μM to 20 μM did not affect the β-oxidation rate, because this would be expected to decrease the free acyl-CoA concentration.

This phenomenon was further investigated in an experiment where mitochondrial β-oxidation was measured at a low, rate-limiting hexadecanoyl-CoA concentration (0.1 μM) in the presence of varying ACBP concentrations to give acyl-CoA/ACBP ratios ranging from 0.05 to 1.0 (Figure 6). The free acyl-CoA concentration can be calculated to vary from 2.3 fM to 67 pM when the molar ratio of acyl-CoA to ACBP was changed from 0.05 to 1. However, the presence of ACBP did not affect the β-oxidation rate. In fact, the β-oxidation rate was decreased when the calculated concentration of free acyl-CoA increased. These results could be interpreted in a number of different ways. One explanation could be that it is the acyl-CoA/ACBP complex which is recognized by the mitochondria and not the free acyl-CoA ester.

The present results are also interesting in regard to the orientation of the CPT1 in the mitochondrial outer membrane (MOM). On the basis of studies with Nargarse at 0 °C, Murthy and Pande (1987) concluded that CPT1 spans the MOM in such

Figure 6 Mitochondrial β-oxidation of [9,10-³H]hexadecanoyl-CoA (0.1 μM) in the presence of 0.1–0.2 μM recombinant bovine ACBP

For experimental details see the Materials and methods section. Data are representative of individual experiments and show means of duplicates ± half the difference.

a way that the binding site for malonyl-CoA is located on the outside and the catalytic acyl-CoA-binding site is on the inside face. Similar results have been obtained by McGarry et al. (1992).

Structural studies (Mannella and Frank, 1984) and reconstitution experiments in lipid bilayers (Freitag et al., 1982; Pinto et al., 1987) suggest that the porin pore in the MOM is only about 2 nm (20 Å) in diameter. The suggested pore length is 50–60 nm (Benz, 1985). The physical dimensions of ACBP–acyl-CoA complex is 2.1 × 3.1 × 3.8 nm (Kragelund et al., 1993). It is therefore very unlikely that the ACBP–acyl-CoA complex can pass through the MOM and donate acyl-CoA to the CPT1 active site on the inner side of the MOM.

The extremely low free concentration of acyl-CoA in the presence of ACBP and the strong detergent nature of these compounds make transport of free acyl-CoA esters by diffusion through pores in the MOM unlikely. Transport of acyl-CoA through the outer-membrane lipid phase in the presence of ACBP is also unlikely, because it has been shown that addition of equimolar amounts of ACBP and hexadecanoyl-CoA completely prevents hexadecanoyl-CoA binding to heat-treated microsomal membranes (Rasmussen et al., 1990). The fact that ACBP also effectively protects hexadecanoyl-CoA from hydrolysis by untreated microsomal membranes (Rasmussen et al., 1993) shows that acyl-CoA esters do not partition into membranes in the presence of equimolar amounts of ACBP.

If it is assumed that ACBP does not pass through the MOM, two possible explanations for the ability of ACBP to donate acyl-CoA to CPT1 therefore exist. The first possibility would be that CPT1 is orientated with both the malonyl-CoA-binding site and the catalytic site on the outer face of the MOM. The second possibility would be that the MOM contains a high-affinity acyl-CoA translocator which can donate acyl-CoA directly to CPT1. The presence of such a translocator has previously been considered by Murthy and Pande (1987). The isolation of a cDNA coding for an ACBP-related possibly membrane-bound protein (Webb et al., 1987) indicates that such a translocator protein might exist.

We thank Erling Knudsen for skilful technical assistance, Bente Meng for typing the manuscript, and Dr. Bent Sigurdskjold, Carlsberg Laboratory, Copenhagen, Denmark, for supplying software and for advice on microcalorimetric measurements. This work was supported by grants from the Danish Natural Science Research Council and the

Protein Engineering Research Center under the Danish Biotechnology Research and Development Program.

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Received 30 July 1993/15 November 1993; accepted 1 December 1993