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Cloning of human acetyl-CoA carboxylase-β and its unique features

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ABSTRACT **Acetyl-CoA carboxylase, which has a molecular mass of 265 kDa (ACC-α), catalyzes the rate-limiting step in the biosynthesis of long-chain fatty acids. In this study we report the complete amino acid sequence and unique features of an isoform of ACC with a molecular mass of 275 kDa (ACC-β), which is primarily expressed in heart and skeletal muscles. In these tissues, ACC-β may be involved in the regulation of fatty acid oxidation, rather than fatty acid biosynthesis. ACC-β contains an amino acid sequence at the N terminus which is about 200 amino acids long and may be uniquely related to the role of ACC-β in controlling carnitine palmitoyltransferase I activity and fatty acid oxidation by mitochondria. If we exclude this unique sequence at the N terminus the two forms of ACC show about 75% amino acid identity. All of the known functional domains of ACC are found in the homologous regions. Human ACC-β cDNA has an open reading frame of 7,343 bases, encoding a protein of 2,458 amino acids, with a calculated molecular mass of 276,638 Da. The mRNA size of human ACC-β is approximately 10 kb and is primarily expressed in heart and skeletal muscle tissues, whereas ACC-α mRNA is detected in all tissues tested. A fragment of ACC-β cDNA was expressed in *Escherichia coli* and antibodies against the peptide were generated to establish that the cDNA sequence that we cloned is that for ACC-β.**

The primary species of mammalian acetyl-CoA carboxylase (ACC) expressed in lipogenic tissues has a molecular mass of 265 kDa (ACC-α)§ (1, 2). ACC-α catalyzes the rate-limiting reaction in the biogenesis of long-chain fatty acids; the reaction product, malonyl-CoA, which is generated only by ACC, is the substrate for fatty acid synthesis. In contrast, although nonlipogenic tissues, such as skeletal and cardiac muscle, in which fatty acids serve as the primary source of energy, contain trace amounts of ACC-α, their predominate form of ACC has a molecular mass of 275–280 kDa (ACC-β) (3–5). In muscle tissue, ACC-β is thought to control fatty acid oxidation by means of the ability of malonyl-CoA to inhibit carnitine-palmitoyl-CoA transferase I (CPT-I), the rate-limiting step in fatty acid uptake and oxidation by mitochondria (3, 6, 7).

Because of the lack of knowledge about the molecular features of ACC-β, the regulatory mechanisms by which fatty acid oxidation in mitochondria is regulated by ACC and malonyl-CoA are largely unknown. Derangements in mitochondrial fatty acid oxidation lead to various diseases, including sudden infant death syndrome, cardiomyopathy, and vascular myopathy (8–10). To explore the role that ACC-β plays in the control of CPT-I and fatty acid oxidation we have cloned human ACC-β cDNA.

ACC-β (11), which is the product of a gene distinct from that for ACC-α (12), has some unusual features, which may be critical to its role in fatty acid oxidation.

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MATERIALS AND METHODS

Materials. Human skeletal muscle cDNA library, human skeletal muscle total RNA, and human multiple tissue Northern blot were purchased from CLONTECH. *Taq* DNA polymerase and restriction enzymes were purchased from Promega; all isotopes were purchased from Amersham. Cell culture media, calf donor serum, and horse serum were purchased from GIBCO/BRL. Streptavidin–alkaline phosphatase conjugate was from Sigma. pCRII T-vector was purchased from Invitrogen. Dulbecco's modified Eagle's medium/F-12 was from GIBCO/BRL.

Cloning of Human ACC Clones. The primers (19-mer) were designed based on the human ACC-α sequence (13), synthesized, and used to isolate human ACC-β clones by polymerase chain reaction (PCR). DNA was purified from human skeletal muscle cDNA library and was used as a template for PCR (1 μg per reaction). The protocol for PCR was the same as described in ref. 13, except for the annealing temperature which varied from 45°C to 60°C, depending on the designed primers. To isolate the initial clone by PCR, we designed one primer (sense sequence) at the biotin-binding site of human ACC-α in such a way that nucleotides encoding Met-Lys-Met were located at the 3′ end of the primer. Using this primer and the other primer, which was designed on the basis of human ACC-α, we were able to isolate the first clone, clone A (base 3037–5518). Clone A shows 70% identity in amino acid sequence in comparison with human ACC-α. Similarly, using one primer from clone A and the other from human ACC-α, we isolated two additional clones, clone B (base 1108–3137) and clone C (base 5439–7784), which overlap the 5′ and 3′ ends of clone A, respectively. The final clone, D (base 1–1174), which contains the first translation initiation codon, was isolated by PCR using one primer from the 5′ end of clone B and another primer for the region of the cloning site of λgt11. PCR products were inserted into pCRII (Invitrogen) and cloned.

Expression of ACC-β Specific cDNA and Antibody Generation. For the construction of expression vector for the 215 amino acids at the N-terminal region of ACC-β (from the initiation methionine to threonine at 215), a 645-bp DNA fragment containing the *Nde*I and *Xho*I sites was amplified through PCR. The amplified PCR products were digested with *Nde*I and *Xho*I and inserted into the plasmid pET-22b(+), which had been digested with the same pair of restriction enzymes. *Escherichia coli* BL21(DE3) was used to overexpress the plasmids. The expressed protein was partially purified using a His-Tag column (Novagen), and partially purified protein was further purified on SDS/8% PAGE. The ex-

Abbreviations: ACC, acetyl-CoA carboxylase; CPT-I, carnitine-palmitoyl-CoA transferase I.

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§In this manuscript, we used the suggested terminology, ACC-α and ACC-β, to avoid designation of these polypeptides by molecular size, as discussed in ref. 11.

pressed ACC-β peptide band was excised and used to generate antibodies in rabbits.

Miscellaneous Procedures. Northern blot analysis (15), Western blot analysis (16), and nick translation (15) were performed as described.

RESULTS

Isolation of Human ACC-β cDNA Clones. Because ACC-β is primarily expressed in muscle tissues, we used DNA purified from human skeletal muscle cDNA library as the template for our PCR reactions in our search for the cDNA coding for ACC-β. We have isolated four clones, as discussed in *Materials and Methods*, whose sequences overlapped to give an 8-kb long cDNA sequence with an open reading frame of 7343 bases, encoding a protein of 2458 amino acids (Fig. 1). The calculated molecular weight is 276,638 Da. All of the clones hybridized with a skeletal muscle RNA species about 10 kb long (Fig. 2).

The major molecular difference between human ACC-α and ACC-β is that the first 217 amino acids of ACC-β include practically no amino acid homology or identity with the first 74 amino acids of ACC-α (13), whereas the remaining amino acid sequences show about 75% identity. Thus, a sequence of about 200 amino acids is unique to ACC-β, and this portion of ACC-β may be involved in functions specific to ACC-β. This aspect is discussed further in the *Discussion*. The molecular weight difference between the two species is largely due to the presence of the extra sequence at the N terminus of ACC-β. Once ACC-α and the ACC-β species are aligned, based on amino acid identity, the functional sites of both species are spaced at approximately the same distances. These functional sites (Fig. 1) include the biotin binding site, the acyl-CoA binding site, the ATP binding site, as well as the enzyme inactivating phosphorylation sites at Ser-79 and Ser-1200 (ACC-α), which are the substrates for 5′-AMP-dependent protein kinase and cAMP-dependent protein kinase, respectively (16). In the case of ACC-β, the Ser-1200 of ACC-α is represented by a threonine residue. It is known that 5′-AMP-dependent protein kinase is more abundant in heart and muscle than in other tissues (17–19), and 5′-AMP-dependent

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MVLLLCLSRLIFSCLTFSWLKIWGKMTDSKPITKSKSEANLIPSQEPFPASDNSGETPQR    60
NGEGHTLPKTPSQAEPASHKGPKDAGRRRNSLPPSHQKPPRNPLSSSDAAHSPELQANGT   120
GTQGLEVTDTNGLSSSARPQGQQAGSPSKEDKKQANIKRQLMTNFILGSFDDYSSDEDSV   180
AGSSRESTRKGSRASLGALSLEAYLTTGEAETRVPTMRPSMSGLHLVKRGREHKKLDLHR   240
DFTVASPAEFVTRFGGDRVIEKVLIANNGIAAVKCMRSIRRWAYEMFRNERAIRFVVMVT   300
PEDLKANAEYIKMADHYVPVPGGPNNNNYANVELIVDIAKRIPVQAVWAGWGHASENPKL   360
PELLCKNGVAFLGPPSEAMWALGDKIASTVVAQTLQVPTLPWSGSGLTVEWTEDDLQQGK   420
RISVPEDVYDKGCVKDVDEGLEAAERIGFSLMIKASEGGGGKGIRKAESAEDFPILFRQV   480
QSEIPGSPIFLMKLAQHARHLEVQILADQYGNAVSLFGRDCSIQRRHQKIVEEAPATIAP   540
LAIFEFMEQCAIRLAKTVGYVSAGTVEYLYSQDGSFHFLELNPRLQVEHPCTEMIADVNL   600
PAAQLQIAMGVPLRRLKDIRLLYGESPWGVTPISFETPSNPPLARGHVIAARITSKNPDE   660
GFKPSSGTVQVLNFRSSKNVWGYFSVAATGGLHEFADSQFGHCFSWGENREEAISNMVVA   720
LKELSIRGDFRTTVEYLINLLKIESFQNNDIDTGWLDYLIAEKVQAEKPDIMLGVVCGAL   780
NVADAMFRTCMTDFLHSLKRGQVLPADSLLNLVDVELIYGGVKYILKVARQSLTMFVLIM   840
NGCHIEIDAHRLNDGGLLLSYNGNSYTTYMKEEVDSYRITIGNKTCVFEKENDPTVLRSP   900
SAGKLTQYTVEDGGHVEAGSSYAEMEVMKMIMTLNVQERGRVKYIKRPGAVLEAGCVVAR   960
LELDDPSKVHPAEPFTGELPAQQTLPILGEKLHQVFHSVLENLTNVMSGFCLPEPVFSIK  1020
LKEWAQKLMMTLRHPSLPLLELQEIMTSVAGRIPAPVEKSVRRAMAQYASNITSVLCQFP  1080
SQQIATILDCHAATLQRKADREVFFINTQSIVQLVQRYRSGIRGYMKTVVLDLLRRYLRV  1140
EHHFQQAHYDKCVINLREQFKPDMSQVLDCIFSHAQVAKKNQLVIMLIDELCGPDPSLSD  1200
ELISILNELTQLSKSEHCKVALRARQILIASHLPSYELRHNQVESIFLSAIDMYGHQFCP  1260
ENLKKLILSETTIFDVLPTFFYHANKVVCMASLEVYVRRGYIAYELNSLQHRQLPDGTCV  1320
VEFQFMLPSSHENRMTVPISITNPDLLRHSTELFMDSGFSPLCQRMGAMVAFRRFEDFTR  1380
NFDEVISCFANVPKDTPLFSEARTSLYSEDDCKSLREEPIHILNVSIQCADHLEDEALVP  1440
ILRTFVQSKKNILVDYGLRRITFLIAQEKEFPKFFTFRAKDEFAEDRIYRHLEPALAFQL  1500
ELNRMRNFDLTAVPCANHKMHLYLGAAKVKEGVEVTDHRFFIRAIIGHSDLITKEASFEY  1560
LQNEGERLLLEAMDELEVAFNNTSVRTDCNHIFLNFVPTVIMDPFKIEESVRYMVMRYGS  1620
RLWKLRVLQAEVKINIRQTTTGSAVPIRLFITNESGYYLDISLYKEVTDSRSGNIMFHSF  1680
GNKQGPQHGMLINTPYVTKDLLQAKRFQAQTLGTTYIYDFPEMFRQALFKLWGSPDKYPK  1740
DILTYTELVLDSQGQLVEMNRLPGGNEVGMVAFKMRFKTQEYPEGRDVIVIGNDITFRIG  1800
SFGPGEDLLYLRASEMARAEAIPKIYVAANSGARIGMAEEIKHMFHVAWVDPEDPHKGFK  1860
YLYLTPQDYTRISSLNSVHCKHIEEGGESRYMITDIIGKDDGLGVENLRGSGMIAGESSL  1920
AYEEIVTISLVTCRAIGIGAYLVRLGQRVIQVENSHIILTGASALNKVLGREVYTSNNQL  1980
GGVQIMHYNGVSHITVPDDFEGVYTILEWLSYMPKDNHSPVPIITPTDPIDREIEFLPSR  2040
APYDPRWMLAGRPHPTLKGTWQSGFFDHGSFKEIMAPWAQTVVTGRARLGGIPVGVIAVE  2100
TRTVEVAVPADPANLDSEAKIIQQAGQVWFPDSAYKTAQAIKDFNREKLPLMIFANWRGF  2160
SGGMKDMYDQVLKFGAYIVDGLRQYKQPILIYIRPMRELRGGSWVVIDATINPLCIEMYA  2220
DKESRGGVLEPEGTVEIKFRKEDLIKSMRRIDPAYKKLMEQLGEPDLSDKDRKDLEGRLK  2280
AREDLLLPIYHQVAVQFADFHDTPGRMLEKGVISDILEWKTARTFLYWRLRRLLLEDQVK  2340
QEILQASGELSHVHIQSMLRRWFVETEGAVKAYLWDNNQVVVQWLEQHWQAGDGPRSTIR  2400
ENITYLKHDSVLKTIRGLVEENPEVAVDCVIYLSQHISPAERAQVVHLLSTMDSPAST    2459
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FIG. 1. Complete deduced amino acid sequence from cDNA of human ACC-β. Mito-sequence (the ACC-β specific N-terminal sequence), boldface type and underlined; the site phosphorylated on ACC-α by 5′-AMP-dependent protein kinase, shaded box; the ATP binding region, open box region; the biotin binding site, underlined; the site phosphorylated on ACC-α by cAMP-dependent protein kinase, filled box; the acyl-CoA binding region, double underline.

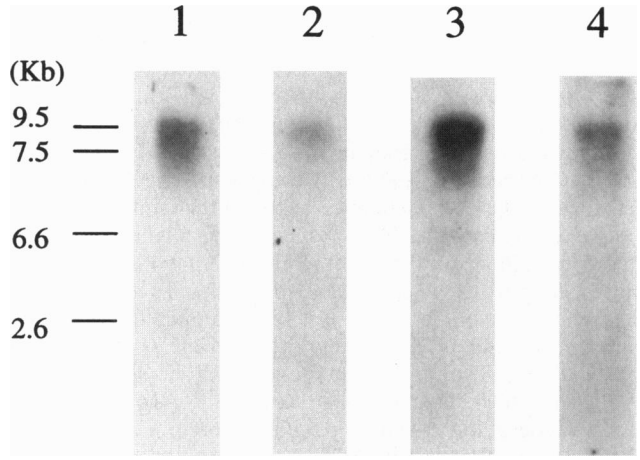

FIG. 2. Northern blot analysis of human ACC mRNA using the individual cDNA clones. Each lane contains 30 μg of human skeletal muscle total RNA and each blot was separately hybridized with the following cDNA as a probe. Lane 1, clone A; lane 2, clone B; lane 3, clone C; lane 4, clone D.

protein kinase may be primarily responsible for phosphorylation and inactivation of ACC-β *in vivo* (20). Numerous possible phosphorylation sites, including those recognized by the cAMP-dependent protein kinase, protein kinase C, and casein kinase II, are found in the N-terminal 200-amino acid sequence of ACC-β. The possible regulatory consequences of phosphorylation in the N-terminal region of ACC-β are discussed in detail in the *Discussion*.

Expression Pattern of Human ACC-β mRNA in Various Human Tissues. The relative levels of expression of the two species of ACC are tissue specific. For example, ACC-β is the predominant species in heart and skeletal muscle tissues, which have only a small amount of ACC-α (3, 4, 11). The reverse is true in the case of lipogenic tissues such as liver and fat tissues. These tissue-specific expression patterns are reflected in the abundance of the corresponding mRNA species (Fig. 3). As shown in Fig. 3*A*, the probe specific for ACC-β hybridized strongly only with 10 kb mRNA from heart and muscle tissue, whereas practically no signals were detected in the samples from liver, pancreas, and kidney at this concentration of total RNA. On the other hand, mRNA species for ACC-α were detected in all samples of tissue tested (Fig. 3*B*). Previously, we

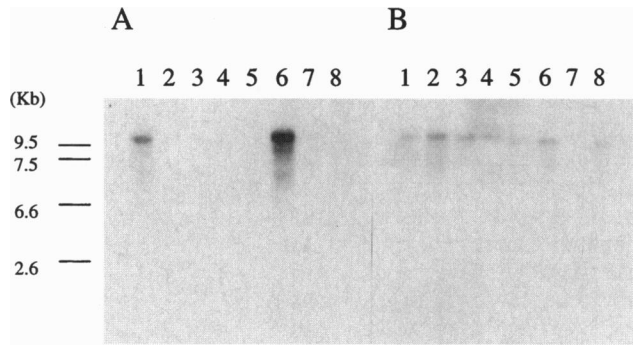

FIG. 3. Expression of human ACC mRNA in various human tissues. Northern blot analysis was performed using the multiple human tissue blot from CLONTECH. (*A*) The blot was hybridized with an ACC-β specific probe. For this experiment, we first generated probes that were specific to each mRNA species. cDNA fragments of ≈600 nucleotides, including the region from amino acids 1338–1470 of ACC-β, as well as the corresponding cDNA fragment of ACC-α, were generated by PCR. (*B*) The same blot was stripped of radioactivity and rehybridized with ACC-α specific probe. Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. Each lane contains 2 μg of poly(A) mRNA.

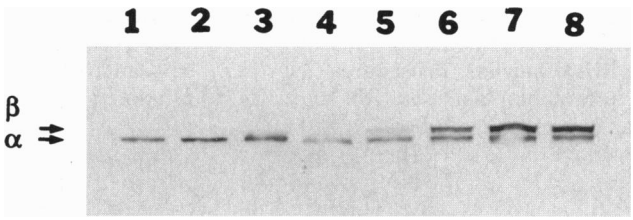

FIG. 4. Western blot analysis of ACC in various cells. Ten micrograms of total protein from different cells were used for Western blot analysis. Streptavidin–alkaline phosphatase obtained from Sigma was used for Western blot analysis (15). Lanes: 1, 30A5 preadipocytes; 2, HepG2; 3, INS-1 β cells; 4, H9c2 cardiomyoblasts; 5–8, H9c2 cells were induced to differentiate (11) for 2, 4, 5, and 6 days, respectively.

indicated that white adipose tissues showed very little ACC-β mRNA, if any (11). The expression patterns of ACC-α and ACC-β in different cell lines are shown in Fig. 4. Lanes 1–4 show that 30A5 preadipocytes (lane 1), HepG2 (lane 2), INS-1 pancreatic β-cell line (lane 3), and H9c2 cardiomyoblasts (lane 4) express only appreciable amounts of ACC-α, but not ACC-β, as examined under these experimental conditions. However, ACC-β was selectively induced when the H9c2 cells were induced to differentiate into myocytes (11). These results, together with the expression of ACC-α and ACC-β in skeletal and heart muscle tissues shown in Fig. 5, indicate that the relative expression patterns of the two species of ACC is tissue specific.

To unequivocally establish that the cDNA that we have cloned is that of ACC-β, we expressed the 215-amino acid peptide at the N terminus, which is specific to ACC-β, and generated antibodies against the peptide. As shown in Fig. 5, the antibodies removed the ACC-β species specifically from the homogenates of mouse skeletal muscle and differentiating muscle cells. Homogenate from mouse skeletal muscle shows primarily the β-form of ACC and a small amount of the α-form (Fig. 5*A*, lane 1). Different antibody preparations removed ACC-β specifically, without affecting the amount of the α-form (Fig. 5*A*, lanes 2–5). In Fig. 5*A*, lane 5, the make-up of ACC in rat H9c2 embryonic cardiomyocytes following 4 days of differentiation is shown. ACC-β was completely removed

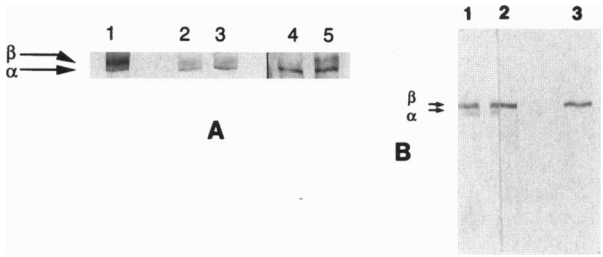

FIG. 5. Effect of antiserum to the mito-sequence on ACC-β immunoprecipitation. (*A*) Mouse skeletal muscle homogenates were treated with different antisera (2 μl) bound to protein A-Sepharose CL-4B (25 μl) as described (21). Following the removal of the precipitates by centrifugation, the supernatants were analyzed by Western blot analysis using streptavidin–alkaline phosphatase conjugates. Lane 1, homogenates that had been treated with control serum; lanes 2 and 3, homogenates treated with antiserum (2 μl) from two different rabbits. H9c2 cells were induced to differentiate for 4 days as described (11). Homogenates were prepared and treated with protein A-Sepharose CL-4B, which had been treated with the control serum (lane 5) or with the antiserum (lane 4). Following centrifugation, the supernatants were examined by Western blot analysis as described above. (*B*) Rat heart homogenates were used for Western blot analysis (15). Lane 1 was probed with streptavidin–alkaline phosphatase. The lane 2 filter was split into two parts and the left half was probed with streptavidin–alkaline phosphatase and the right half with anti-ACC-β. Lane 3 was probed with anti-ACC-β.

when the homogenate of differentiated muscle cells was analyzed following the treatment with the antibodies against ACC-β. As shown in Fig. 5*B*, where a Western blot analysis was carried out, our antibodies also crossreacted specifically with the ACC-β in the homogenates of rat heart (Fig. 5*B*, lane 2). When antibodies against streptavidin were used, both ACC-α and ACC-β were identified (Fig. 5*B*, left half of lane 2 and lane 1), whereas our antibodies only identified ACC-β (Fig. 5*B*, right half of lane 2 and lane 3). Because antibodies were generated by the peptide whose coding sequence was based on the cloned cDNA, this result established that the cDNA sequence is for ACC-β.

DISCUSSION

The general belief that ACC plays a role in the control of fatty acid oxidation was based on the following observations. First, CPT-I, an essential component of fatty acid oxidation, is extremely sensitive to malonyl-CoA inhibition (6, 7, 22, 23) and malonyl-CoA is only generated by ACC. Second, muscle tissues are non-lipogenic and yet contain a large amount of ACC. Recently, the ACC in these tissues was found to be mostly the ACC form with a molecular mass of 275–280 kDa (3, 24, 25). Physiological conditions that cause a decrease in ACC activity and malonyl-CoA levels are accompanied by accelerated rates of fatty acid oxidation in liver (26) and heart (20, 24, 27).

CPT-I, which is located in the inner phase of the outer membrane of the mitochondrion (28), catalyzes the rate-limiting step in fatty acid uptake and fatty acid oxidation. McGarry *et al.* (7), and other groups, established that CPT-I is particularly sensitive to malonyl-CoA inhibition (7). It is obvious that the mechanism by which the concentration of malonyl-CoA near the malonyl-CoA binding component of CPT-I is controlled must be a vital aspect of the regulation of fatty acid oxidation. The malonyl-CoA binding component of CPT-I faces the outer phase (the cytosolic sphere) of the outer membrane of the mitochondrion (28), whereas the catalytic region of CPT-I faces the inner phase of the outer membrane (28). Thus, ACC-β would not have to be imported into the inner phase to control CPT-I activity.

The N-terminal sequence of about 200 amino acids unique to ACC-β is rich in hydroxy amino acids (46 serines and threonines) and basic amino acids (25 arginines and lysines). Such an amino acid composition is typical of transit peptides that target to mitochondria and chloroplasts (29). However, this sequence is frequently broken by proline residues (17 prolines between residues 31 and 147). Although there are abundant basic amino acids, they are counterbalanced by a comparable number of acidic residues (23 glutamic and aspartic acids). Not only is the whole sequence comparatively long, but the above features would make it difficult to form the amphiphilical secondary structures that are found in the mitochondrial import proteins. Overall, the unique sequence exhibits strong hydrophilic features, except for the first 25-amino acid portion of the N terminus, which contains a hydrophobic region surrounded by positive charges, a typical feature of mitochondrial signal peptides. However, this region does not form the ideal amphiphilical secondary structure that is required for mitochondrial import proteins.

Our hypothesis is that this region of ACC-β may be required for targeting and/or anchoring to the mitochondrial outer membrane in such a way as to control the malonyl-CoA regulatory site of CPT-I, which faces the cytosolic side of the outer membrane of the mitochondrion (28). This hypothesis is illustrated in Fig. 6. In this diagram the ACC-β-specific N terminal sequence is designated as the mito-sequence. The hydrophobic region of ACC-β (the first 25 amino acids) may be responsible for binding or anchoring into the outer membrane of the mitochondrion. This could control CPT-I activity

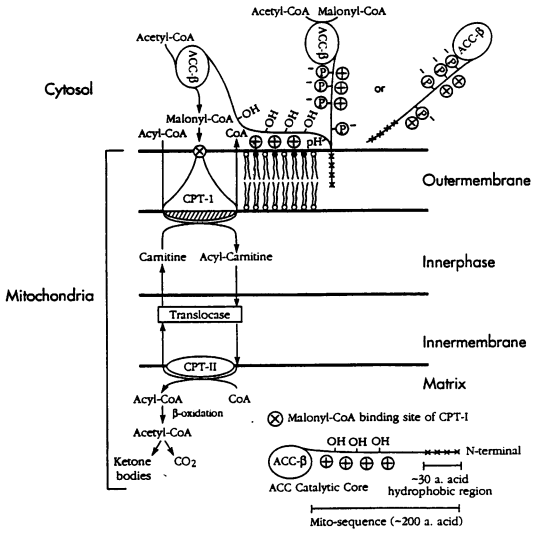

FIG. 6. Role of ACC-β in the control of CPT-I. In this model, the N-terminal hydrophobic region of the mito-sequence is responsible for anchoring or binding of ACC-β in such a way as to control malonyl-CoA concentration near the malonyl-CoA binding site of CPT-I. Phosphorylation/dephosphorylation of the hydroxy amino acid residues in the mito-sequence may control the binding or anchoring of ACC-β to the outer membrane of the mitochondrion.

by generating malonyl-CoA at or near the malonyl-CoA binding site of CPT-I. Our second hypothesis concerning this model is that the phosphorylation of the mito-sequence at serine and threonine residues could control ACC-β binding or anchoring to the membrane by increasing negative charges on the mito-sequence. In the absence of phosphorylation, the basic amino acids on the mito-sequence would interact with the negative charges of the membrane lipids and augment the hydrophobic interaction between the hydrophobic region of ACC-β and the outer membrane. This model is currently being experimentally tested. Such interactions could increase the local concentration of a metabolite as much as 1000-fold (30).

Allred and coworkers have reported that some species of ACC do bind to the outer membrane of liver mitochondria reversibly, as much as 75%, depending upon the physiological condition of the rats (31, 32). However, the association of ACC-β with mitochondria has not been easily demonstrated (33, 34). It is possible that proteolytic cleavage of the N-terminal segment might occur during subcellular fractionation, releasing the catalytic domain of ACC-β to the cytosol.

mRNAs for the two forms of ACC occur in all tissues that we have examined, although their relative amounts are tissue specific. In addition, these two enzymes catalyze the same reaction. mRNAs of both forms of ACC are about 10 kbp long and the cloning and sorting of cDNA fragments have been extremely difficult. We have previously reported a cDNA sequence for human ACC-α, which is a hybrid molecule of the ACC-α and ACC-β forms (13). A recent report of a different version of the human ACC sequence (14) prompted us to reexamine the alignment of different ACC clones and to perform additional cloning to clarify the nature of the human ACC cDNA clones. It is clear that the human cDNA sequence reported by Abu *et al.* (14) is that of ACC-α. Those clones isolated from the cDNA library, which was constructed using the total RNA of human white adipose tissue (13), contained the sequences for ACC-β, whereas the sequences obtained by PCR using HepG2 RNA represented those of ACC-α. Although the white adipose tissue or 30A5 preadipocytes show no detectable amounts of ACC-β mRNA or protein, the cDNA library, which was constructed using the white adipose tissue, contained readily detectable cDNA clones (13). However, these clones were restricted to those representing about a 3-kb

long fragment for the 3′-end (11, 13). These observations indicate that ACC-β is expressed in all cells including adipose tissues. If ACC-β is indeed involved in the control of mitochondrial fatty acid oxidation, it is reasonable to expect that ACC-β is expressed in all cells.

The sequence reported here is for ACC-β. Its gene has been localized to chromosome 12 (11), whereas the ACC-α gene is on chromosome 17 (12).

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