Identification of an Isozymic Form of Acetyl-CoA Carboxylase*

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Acetyl-CoA carboxylase (ACC) is a major rate-limiting enzyme of fatty acid biosynthesis; its product, malonyl-CoA, also contributes to the regulation of fatty acid oxidation and elongation. Using monospecific antibodies directed against rat liver ACC and N- and C-terminal antipeptide antibodies raised against predicted sequences of the cloned ACC of $M$, 265,000, we have identified a unique biotin-containing cytosolic protein of molecular mass 280,000 daltons that is distinct from this 265,000-dalton protein. This protein is uniquely expressed in rat cardiac and skeletal muscle and is co-expressed with the 265,000-dalton protein in rat liver, mammary gland, and brown adipose tissue. In the fed rat, white adipose tissue content is unaltered by nutritional manipulation. Avidin-Sepharose isolates of citrate-dependent ACC from the heart reveal only the 280,000-dalton protein, while white adipose tissue isolates show only the 265,000 form. These species differ in the sensitivity to citrate activation and in the $K_m$ for acetyl-CoA. Antibodies reactive with the 280,000-dalton protein on immunoblotting precipitate ACC activity in heart isolates, while white adipose ACC is precipitated only by antibodies specific for the 265,000-dalton species. However, in ACC isolates where both proteins are present, a heteroisoenzmye complex can be detected by immunoprecipitation and by a sandwich enzyme-linked immunosorbent assay. We conclude that the 280,000-dalton protein is an isozyme of ACC, distinct from the previously cloned 265,000-dalton species. Its presence in cardiac and skeletal muscle, where fatty acid synthesis rates are low, suggest that it might play alternative roles in these tissues such as regulation of fatty acid oxidation or microsomal fatty acid elongation.

Acetyl-CoA carboxylase (EC 6.4.1.2) is a rate-limiting enzyme of fatty acid biosynthesis in liver, adipose tissue, and mammary gland. The product of its reaction, malonyl-CoA, is important not only as the immediate precursor of fatty acid synthesis, but for the regulation of fatty acid oxidation (through modulation of mitochondrial entry of long-chain fatty acyl-CoA) (1-3) and as a primer for fatty acid elongation in the microsomes. ACC is regulated both through short-term mechanisms (seconds to minutes) as a result of altered covalent phosphorylation and allosteric regulation and through long-term mechanisms (hours to days) due to changes in enzyme content, perhaps through alterations in gene transcription rates (4-9). Thus, it has been an object of intense study in several laboratories attempting to discern the molecular mechanisms involved in its regulation. Two laboratories have reported the isolation of cDNAs for ACC (chicken liver and rat liver; cloned initially as partial sequences and the total coding sequence completed by overlap oligonucleotide screening) and these cDNAs have been completely sequenced, predicting a polypeptide chain of 2345 amino acids (rat liver; corresponding to the known peptide chain of 265,000 daltons) and containing several recognizable functional domains, including a biotin site, a CoA binding site, likely ATP/HC03-binding site and a cluster of phosphorylation sites (10-11). All work to date on ACC has assumed that there is one species of the enzyme expressed in several different tissues and that the regulation of this enzyme is responsible for its multiple catalytic roles.

Based on the present work, we report that there is at least one recognizable isozyme of ACC with different tissue distribution and long-term regulation from the previously characterized isoform. Evidence for its existence is derived from specific immunoreactivity, enzyme isolation, immunoprecipitation, and enzyme kinetics.

EXPERIMENTAL PROCEDURES

Methods

Isolation of Acetyl-CoA Carboxylase—ACC was isolated from various rat tissues by immunospecific avidin-Sepharose chromatography (12). Male Sprague-Dawley rats (130-150 g) were used in all these experiments. Rats were either maintained on normal rat chow (fed) or fasted for 48 h (fasted), or fasted for 48 h followed by 48 h of feeding with a high carbohydrate chow (Bioserv) (fasted/refed). Tissues were removed, rinsed briefly in PBS, and immediately frozen in liquid nitrogen. Tissues were then homogenized in Buffer A (Tris-Cl (50 mM; pH 7.5 (4 °C)), sodium fluoride (0.1 M), EDTA (1 mM), $\beta$-mercaptoethanol (10 mM), sucrose (0.25 M), and seven protease inhibitors (12). ACC was then isolated from cytosolic fractions after 40% NH4SO4 fractionation.

Tissue Sampling for Immunoblot Analysis—For analysis of ACC protein content in various tissues, rat tissues were removed, rinsed briefly in PBS, and immediately frozen in liquid nitrogen. They were then homogenized in buffer A (1:3, w/v). Protein concentration was determined by the Lowry method.

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determined on the total homogenate and matched for all tissues. Samples were then prepared for SDS-gel electrophoresis, as described previously (13). After boiling of the gel sample, they were then trituated through a 25-gauge needle prior to gel loading. In these experiments, in addition to male rats we also used lactating female Sprague-Dawley rats and Balb/c mice (both normally fed) for analysis of the liver ACC.

Antibody Preparation and Characterization Five antibodies were employed in the present study. The first was raised in sheep immunized with ACC from fasted/refed rat liver isolated by a technique that did not include avidin-Sepharose chromatography (14). The second was a panel of monoclonal anti-rat liver ACC antibodies against avidin-Sepharose purified fasted/refed rat liver enzyme (15); one of these antibodies of the IgG subclass (1\'A2D) was chosen for use in this study because of its specific reactivity. A third polyclonal antibody was raised in rabbits against fasted/refed rat liver ACC where the major staining band (265,000 daltons) was eluted from an SDS gel for use as an immunogen. This band was separated on a 5% SDS-acrylamide gel, identified by KCl staining, and the region of staining excised. The gel slices were homogenized in a Dounce homogenizer in 0.1% SDS, allowed to sit overnight, and centrifuged. The supernatant was removed and the slices rehomogenized and resuspended. The combined supernatants were mixed with Freund's complete adjuvant for primary immunization; boosting injections were performed after 3 and 6 weeks with the SDS extract mixed in incomplete Freund's after 4 and 6 weeks followed by an exsanguination.

Purification of Other Tissues. Sprague-Dawley rats and Balb/c mice (both normally fed) for analysis of other tissues.

All polyclonal antibodies and non-immune serum were further purified to the IgG fraction by DE-AGffi-Gel Blue chromatography. Reactivity with specific proteins was assessed by immunoblotting (see "Results").

Immunoblotting and Gel Electrophoresis--Immunoblotting was performed on purified ACC and samples from tissue homogenates after electrophoresis on 5% SDS-acrylamide gels (15) followed by transfer to nitrocellulose and antibody probing, as in Ref. 15. Protein A was used as the detecting antigen for the polyclonal antibodies and 125I-goat anti-mouse IgG for the monoclonal antibody. Radiography was performed at -70 °C using pre-flashed XAR film (Kodak) with an enhancing screen.

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enzyme velocity and the kinetic constants were obtained in assays with either product (not shown). All other reagents were purchased from Sigma.

RESULTS

Identification of a 280,000-Dalton Protein in Purified Acetyl-CoA Carboxylase by Protein Staining and Immunoblotting—Acetyl-CoA carboxylase, purified from the cytosolic fraction of the livers of fasted/refed rats by monomeric avidin-Sepharose chromatography, shows two high molecular mass proteins on Coomassie staining of 280,000 and 265,000 daltons on 5% acrylamide-SDS gels (Fig. 1; left panel). A minor contaminant at molecular mass 130,000 daltons is also seen and is likely pyruvate carboxylase, which arises from mitochondrial disruption during tissue homogenization. By gel scanning, the 280,000-dalton component constitutes approximately 10–15% of the total mass of the two high molecular mass proteins. A similar pattern of Coomassie staining is also observable in ACC isolated from fed rat liver and rat lactating mammary gland (not shown).

The immunoreactivity of the 280,000- and 265,000-dalton components was determined by immunoblotting with five different antibody preparations. As shown in Fig. 1 (right panels; even-numbered lanes), isolated ACC from fasted/refed liver shows variable reactivity with these antibodies. The sheep polyclonal antibody (SPC) recognizes both proteins, while the other antibodies are monospecific. The 280,000-dalton protein is recognized only by the monoclonal antibody (7AD3), while the 265,000-dalton protein is exclusively recognized by the antibodies raised against the N- and C-terminal peptides (N and C) and the rabbit polyclonal antibody. These latter data with the anti-peptide antibodies suggest strongly that the 265,000-dalton species corresponds to the predicted sequence of the previously cloned form of ACC (11); however, the 280,000-dalton protein lacks these N- and C-terminal epitopes. The odd-numbered lanes in this right panel of Fig. 1 show reactivity with ACC isolated from the livers of normally feeding rats; the same pattern of immunoreactivity is observed and the ratio of the 280,000/265,000-dalton components is the same as with the fasted/refed enzyme. The immunostaining with the sheep polyclonal antibody would seem to indicate a different mass ratio of the two species than that observed by direct protein staining. However, in the polyclonal IgG preparation, antibodies of varying affinity for the two species likely exist, accounting for this difference. Since the same ratios (on immunostaining) are seen in crude tissue homogenates (see below), differential recovery of the two species on avidin-Sepharose chromatography due to differences in biotin content or in avidin affinity cannot account for the ratio observed on immunoblotting of purified preparations.

Demonstration of the Presence of Protein Biotinylation by Immunoblotting—Since they were isolated by highly specific avidin-Sepharose affinity chromatography, both the 280,000- and 265,000-dalton proteins should contain biotin. It was important, however, to directly demonstrate that biotin was present in both species, since it is possible that non-biotin-containing proteins might be isolated by this technique, if they bound tightly to a biotin-containing species. Accordingly, SDS-denatured samples of fasted/refed ACC were incubated with different concentrations of avidin prior to SDS-gel electrophoresis and immunoblotting. Because of the high affinity of avidin for biotin (K_D = 10^{-14} to 10^{-15} M), avidin will remain bound to biotin-containing proteins even in the presence of SDS. As shown in Fig. 2, the binding of avidin increases the molecular mass in a concentration-dependent manner of both the 265,000- and 280,000-dalton proteins (as recognized by sheep polyclonal antibody) and the 280,000-dalton protein as probed with 7AD3 monoclonal antibody. These data provide firm evidence that both high molecular weight cytosolic proteins contain biotin. Paretologically, the immunoreactivity also provides evidence that ACC prepared by avidin-Sepharose chromatography does not contain apoenzyme (present potentially by association with the biotin-containing ACC).
since all immunoreactivity observable shifts to a higher molecular weight on avidin binding.

**Tissue Distribution of the 280,000- and 265,000-Dalton Proteins**—In order to assess the tissue distribution of the 280,000- and 265,000-dalton proteins, tissue homogenates from normally feeding rats were matched for total protein content and prepared for gel electrophoresis and immunoblotting with the five antibodies. As shown in Fig. 3 (right panel), both proteins are present in liver and brown adipose tissue, while white adipose tissue contains only the 265,000-dalton protein and the heart (and skeletal muscle; not shown in this panel) contain only the 280,000-dalton protein. Neither protein was detectable in spleen, kidney, intestine, lung, or brain (latter not shown). The distribution of the 280,000-dalton protein was confirmed on immunoblotting with the 7AD3 antibody (Fig. 3; left panel). This protein is absent from white adipose tissue, but present in liver, brown adipose tissue, heart, and skeletal muscle.

The absence of the 265,000-dalton protein in rat heart and skeletal muscle was confirmed by immunoblotting with the antibodies raised against the N- and C-terminal domains of the predicted ACC sequence of the 265,000-dalton protein and with the rabbit polyclonal antibody (Fig. 4). Liver, white adipose tissue, and brown adipose tissue all contain the 265,000-dalton protein, while none could be detected with any of these antibodies in rat heart and skeletal muscle homogenates.

On analysis of homogenates from other tissues analyzed similarly (not shown), both proteins are found to be present in lactating mammary gland and Reuber hepatoma cells, but rat testes, bladder, uterus, and placenta contain only the 265,000-dalton protein. There is no discernable difference between male and female rats in the relative mass of the two species in liver, heart, white adipose tissue, and skeletal muscle extracts. In the mouse, liver and adipose tissue contain a protein of 265,000 daltons, while no 280,000-dalton protein could be identified in any murine tissues. Given species differences, it is possible that any of these murine tissues might contain a similar 280,000-dalton protein that does not react with either the monoclonal or sheep polyclonal antibody. Three murine cell lines, B16F1 monocytes, G8 myoblasts, and 3T3-L1 adipocytes also display no 280,000-dalton protein, although each protein contains a immunoreactivity similar to the 265,000-dalton protein.

**Protein Content with Altered Nutritional State and in Subcellular Fractions**—Acetyl-CoA carboxylase content in liver is well known to be subject to nutritional regulation, increasing on feeding fasted rats a high carbohydrate diet and diminishing on fasting. The effect of this nutritional manipulation on the content of the 280,000- and 265,000-dalton species was therefore studied in rat heart and white adipose tissue homogenates and compared to that in liver homogenates from the same animals (Fig. 5). As determined by immunoblotting with the sheep polyclonal antibody, the anti-N-terminal antibody and the monoclonal ACC 7AD3, liver homogenates show the induction and repression of both species. White adipose tissue in the fed state shows only the 265,000-dalton species that declines to a small extent during fasting and increases on fasting/refeeding. Traces of the 280,000-dalton protein, however, do appear in this tissue on fasting/refeeding (as detected with the monoclonal antibody). In contrast to these tissues, the content of the 280,000-dalton protein in
Heart (and skeletal muscle; not shown) is unaltered by these nutritional manipulations.

It has been suggested that nutritional alterations might alter the association of ACC with the mitochondrial fraction and that there might be interchanges of ACC between this fraction and the cytosol as a function of altered nutrition or diabetes mellitus (16, 22). However, we have found that both proteins are nearly entirely cytosolic in distribution, as judged by immunoblotting of cytosol, washed mitochondria, and microsomes after fractionation of liver and do not differ in their distribution in livers derived from fasted, fed, or fasted/refed (not shown).

Acetyl-CoA Carboxylase Activity: Isolation and Immunoprecipitation—While the above data clearly show that the 280,000-dalton protein is distinct from the 265,000-dalton protein by immunoreactivity, tissue distribution, and nutritional modulation and that the 265,000-dalton protein likely corresponds to the previously recognized form of ACC (by deduced molecular mass and N- and C-terminal antibody reactivity), evidence that the 280,000-dalton protein was indeed an isozyme of acetyl-CoA carboxylase was derived from enzyme isolation and immunoprecipitation. ACC was isolated from adipose tissue and heart of normally feeding rats by monomeric avidin-Sepharose chromatography. Both eluates show a peak of ACC activity that is nearly entirely citrate-dependent (Fig. 6; left panel). The peak fractions from these eluates were examined by immunoblotting with three antibodies. As shown in the right panel of Fig. 6, adipose ACC reacts only with the N-terminal antibody and the sheep polyclonal antibody (the latter corresponding the 280,000-dalton species; hereafter called ACC 280), while heart ACC reacts exclusively with the monoclonal 7AD3 and the sheep polyclonal antibody (corresponding to the 280,000-dalton species; hereafter called ACC 280).

Immunoprecipitation of isolated white adipose tissue ACC shows that citrate-dependent ACC activity is removed by the N-terminal antibody, but none is removed by the monoclonal ACC 7AD3 (Fig. 7). In contrast, only the monoclonal precipitates ACC activity from an isolated rat heart preparation, while no activity is removed on incubation with the N-terminal antibody. We have been unable to completely precipitate all activity under these incubation conditions with variable amounts of either antibody in both preparations of ACC, although complete precipitation can be achieved by immobilizing the antibodies on protein A-Sepharose beads by cross-linkage.2

Demonstration of a Heteroisozyme Complex—Immunoprecipitation of isolated native fasted/refed liver enzyme with ACC 280-specific antibody (ACC 7AD3) and ACC 265-specific antibody (anti-N-terminal) reveals that both antibodies precipitate ACC activity (Fig. 8A). However, on immunoblotting, the precipitation of ACC 280 by anti-N-terminal antibody and precipitation of ACC 265 by ACC 7AD3 is observed (Fig.

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2 A. Iverson and L. A. Witters, unpublished observations.
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FIG. 8. Immunoprecipitation of fasted/refed liver enzyme: analysis by enzyme activity and immunoblotting. Shown in A (left) is ACC activity precipitated from 200 ng of fasted/refed rat liver enzyme by control monoclonal (P3), anti-N-terminal antibody (N), and ACC 7AD3 monoclonal (M), as detailed under "Methods." Total milliunits represents activity not subjected to the precipitation procedure, but allowed to sit at 4°C during the procedure. The amount precipitated (ppt) with antibodies in each instance was calculated as the difference between this activity and the amount remaining in the supernatant after immunoprecipitation. Shown in B (right) is a corresponding immunoblot of equal volumes of immunosupernatants (S) and immunopellets (resolubilized in a volume identical to that of the supernatant; P), as analyzed on a 5% SDS-acrylamide gel with electrophoretic transfer to nitrocellulose and probing with sheep polyclonal antibody. The pattern of immunoprecipitation of each isozyme was additionally confirmed on immunoblots individually probed with the anti-N-terminal and ACC 7AD3 antibodies (not shown).

FIG. 9. Sandwich ELISA of various ACC preparations. Shown are the results of the sandwich ELISA employing ACC 7AD3 as the trapping antibody and the anti-N-terminal antibody as the detecting antibody, as detailed under "Methods." The data are depicted as absorbance at 415 nm (corrected for blanks) after 15 min development (determined to be linear with respect to liver ACC load). Five-hundred ng of fasted/refed (F/R) liver, fed liver, and mammary enzyme were analyzed in triplicate wells. The actual ACC protein loads of brown adipose tissue (BAT), white adipose tissue (adipose), heart, and skeletal muscle (SM) could not be determined due to their dilution into 2 mg/ml bovine serum albumin on isolation (added to prevent loss of activity and protein), so they were analyzed based on milliunit activity equated to that of the fasted/refed liver sample. Given likely variations in actual specific activity, they are therefore not likely loaded at the same ACC protein concentration. However, in this assay, detectable absorbance of the fasted/refed liver enzyme complex can be seen at <100 ng of total enzyme protein, so that it is likely that this method of sample matching (which would then encompass a 35-fold range of specific activity) should be able to detect sandwiched antigen, if present.

8B). These later data are consistent with association of the two isozymes in the native state.

Further evidence for this association was obtained with a sandwich ELISA employing ACC 7AD3 antibody as the trapping antibody and the anti-N-terminal antibody as the detecting one. With liver (fasted/refed and fed), mammary, and brown adipose tissue enzyme, apparent complexes of ACC 280 and 265 are readily detectable, while no complexes (as expected) are detectable in adipose, heart, and skeletal muscle preparations (Fig. 9). An identical result is obtained if the anti-C-terminal antibody is used as the detecting antibody (results not shown). In the reverse sandwich (using the antibodies in the other orientation with peptide-affinity purified anti-N-terminal as the trapping antibody and ACC 7AD3 as the detecting antibody), complexes can also be detected. We have also developed a sandwich ELISA with peptide-affinity purified N-terminal antibody as the trapping antibody and biotinylated N-terminal antibody as the detecting one and can easily detect enzyme species containing multiple copies of ACC 265. However, we have been unable to detect species in liver that contain only ACC 280.

Enzyme Kinetics—ACC 280 from heart and skeletal muscle and ACC 265 from white adipose tissue can also be distinguished by both citrate and acetyl-CoA kinetics, as determined with enzyme purified from each tissue. The apparent $K_{m}$ for citrate (derived from linear Eadie-Hofstee plots) is greater for ACC 280 (heart: 2.3 mM; skeletal muscle: 2.0 mM; mean of two preparations of each) than that of ACC 265 (white adipose tissue 0.64 ± 0.09 mM [mean ± S.D. of three preparations]). ACC 280 also displays a higher $K_{m}$ for acetyl-CoA (heart: 180 ± 40 μM [three preparations]; skeletal muscle: 167 μM [two preparations], white adipose tissue 74 ± 7 μM [three preparations]). No differences have been noted in either ATP/Mg$^{2+}$ or bicarbonate kinetics. In ACC isolates from tissues with both isozymes, the kinetics are non-linear with respect to both citrate and acetyl-CoA, indicating heterogeneity of enzyme species (not shown). A detailed description of these kinetic analyses will appear elsewhere.
DISCUSSION

The results of the present investigation demonstrate an isozyme of acetyl-CoA carboxylase that displays differential immunoreactivity, tissue distribution, and nutritional regulation from that of the previously recognized form of the enzyme. Several lines of evidence suggest that it is a unique enzyme. First, ACC 280, a biotin-containing cytosolic protein, is not recognized by antibodies raised against the N-terminal and C-terminal peptide residues of the predicted ACC 265 sequence (10, 11). An additional rabbit polyclonal antibody additionally recognizes only the ACC 265; this antibody does not recognize the N- and C-terminal peptides (results not shown) implying the recognition of other epitopes in ACC 265, which are missing from ACC 280. These results, taken together, indicate that ACC 265 cannot be generated from the ACC 280 by proteolysis, since such an event would have to add the terminal epitopes of the N- and C-terminal peptides, the epitopes of the rabbit polyclonal antibody and delete the monoclonal epitope. Furthermore, ACC 280 is very unlikely to be a differently migrating species on SDS gels because of post-translational modification. In order to generate ACC 280 by these mechanisms, ACC 280 would have to gain ACC 7AD3 reactivity, while simultaneously losing reactivity to the N- and C-terminal antibodies and the rabbit polyclonal antibody, a very unlikely occurrence. The relative amounts of ACC 265 and ACC 280 in the fed liver (a highly phosphorylated form of ACC) and the fasted/refed liver (a highly dephosphorylated form of ACC) (23, 24) are the same, indicating that the phosphorylation state is not responsible for the observed heterogeneity in molecular weight on SDS gels.

Second, ACC activity is immunoprecipitated by ACC 7AD3 from purified heart ACC preparations, but not from white adipose tissue isolates (which contains only ACC 265), the latter being precipitable by the N-terminal directed antibody. Third, monoclonic avidin-Sepharose isolated of heart and white adipose ACC, which both contain typical citrate-dependent ACC activity, contain only ACC 280 and ACC 265, respectively. Fourth, tissue surveys by immunoblotting of tissue extracts from several rat tissues indicate differential distribution of ACC 280 and ACC 265.

Fifth, ACC 280 and ACC 265 are recognizably different in terms of tissue expression after nutritional manipulation of the rat. In the liver, the content of both ACC 280 and 265 content is reduced after a 48-h fast and both “superinduce” after an additional period of feeding for 48 h with a high carbohydrate diet. There is a qualitatively similar changes in white adipose tissue with these nutritional manipulations; although ACC 265 is the predominant ACC protein in all three nutritional states, traces of 280 do appear on fasting/refeeding. In the heart (and skeletal muscle), however, ACC 280 content is unchanged by either fasting or fasting/refeeding unaccompanied by the appearance of ACC 265, as compared to normally feeding control animals. The regulation of ACC 280 content is thus under tissue-specific control. This could represent the effects of different tissue-specific promoters of a ACC 280 gene, as has been suggested for ACC 265 (25) or due to tissue-specific alternative splicing that varies as a function of the nutritional state in different tissues. ACC 280, as identified in this study by immunologic reactivity, could, in fact, represent several different ACC isoforms (with different genes and promoters) in the tissues in which it is present; there is certainly adequate precedent for many onzymes having different isozymic forms in, for example, skeletal muscle, heart, and liver.

Lastly, ACC 280 has distinctly different kinetic properties than ACC 265 with a higher activation constant for citrate and higher $K_m$ for acetyl-CoA. Whether this reflects differences in primary protein structure or in post-translation modification (for example, varying degrees of phosphorylation) remains to be determined.

Others have reported the existence of two high molecular weight cytosolic biotin-containing species in rat liver as determined by [14C]avidin binding (16, 22), but these proteins have not heretofore been characterized as to their actual ACP activity, their relationship to one another, and their tissue distribution. Multiple species of ACC 265 mRNA have also been reported (25), but these structural differences appear to lie exclusively in the 5′-untranslated region with only a single start site for the translated sequence; this latter observation coupled with the lack of immunoreactivity demonstrable in the present study of ACC 280 with the predicted N-terminal domain of the deduced structure of ACC 265 would seem to indicate that ACC 280 arises from a unique mRNA. Whether ACC 280 is a product of a second ACC gene or arises via tissue-specific alternative mRNA splicing of a single primary transcript for both ACC 280 and ACC 265 is not ascertainable in the present work. Although ACC 265 and ACC 280 are likely homologous/identical in several important catalytic domains, splicing that would generate immunoheterogeneity would have to be extensive, variably adding or deleting the N- and C-terminal 15 amino acids, the epitopes recognized by the rabbit polyclonal antibody and the epitope recognized by the monoclonal 7AD3.

ACC exists as enzyme protomer (13-16 S) consisting of two noncovalently associated identical polypeptide chains and oligomers/polymers that represent linear arrays of protomer subunits of up to 40-50 S, the formation of the latter being favored by citrate and perhaps by dephosphorylation (29, 29–29). In tissues such as liver and mammary gland where at least two isozymes are now known to be present, there appears to an association of the two isozymes in a complex, as demonstrated by immunoprecipitation and ELISA. It seems possible that a multiplicity of forms of total ACC might be present in these tissues including heterovertomers and heteropolymers, in addition to protomers and polymers with a single polypeptide subunit. Several factors, including allosteric regulation and variable degrees of phosphorylation of either isozyme, might determine this noncovalent association. In this regard, it is of interest to note that the amount of measurable heteroisoyme complex in fed liver ACC (a highly phosphorylated form (24)) is less than that in fasted/refed ACC (dephosphorylated), despite an identical mass ratio in the total isolate (Figs. 1 and 9). The physical nature of these complexes, the factors underlying their composition and their impact on enzyme activity are currently under investigation.

The exact roles of ACC 280 cannot be clarified in the present study. In tissues where both isozymes are expressed (liver, hepatoma cells, mammary gland, and brown adipose tissue) it may contribute significantly to the overall ACC catalytic activity forming malonyl-CoA as a substrate for fatty acid synthesis, for the regulation of fatty acid oxidation, or for fatty acid elongation. In tissues where only ACC 280 is expressed (heart, skeletal muscle) in which fatty acid synthesis rates are low, we speculate that it might play a more important role in regulating fatty acid oxidation (via its product malonyl-CoA, whose content allosterically regulates carnitine palmitoyltransferase (1–3)), since these tissues have high oxidative rate for this substrate and utilize fatty acid preferentially under certain circumstances. Interestingly, brown adipose tissue (a mitochondrial-rich tissue, like heart...
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and skeletal muscle), which has the highest content of ACC 280 (Fig. 3), also has high fatty acid oxidative rates.

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REFERENCES
27. Moss, J., Yamagishi, M., Kleinschmidt, A. K., and Lane, M. D. (1972) Biochemistry 11, 3779-3786
Identification of an isozymic form of acetyl-CoA carboxylase.
A Bianchi, J L Evans, A J Iverson, A C Nordlund, T D Watts and L A Witters


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