Structure and Regulation of Rat Long-chain Acyl-CoA Synthetase*

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Complementary DNAs encoding rat long-chain acyl-CoA synthetase have been isolated. The cDNAs were identified using synthetic oligonucleotide probes based on partial amino acid sequences of lysyl endopeptidase peptides of the purified enzyme. Rat long-chain acyl-CoA synthetase is predicted to contain 699 amino acid residues and to have a calculated molecular weight of 78,177. Significant sequence similarity was found between parts of long-chain acyl-CoA synthetase and firefly luciferase. Based on the similarity of the reaction mechanisms of the two enzymes, we propose a function for the similar region. The long-chain acyl-CoA synthetase mRNA is expressed in liver, heart, and epididymal adipose tissues and, to a much lesser extent, in brain, small intestine, and lung. The level of long-chain acyl-CoA synthetase mRNA is increased 7-8-fold in rat liver by feeding a diet high in carbohydrate or fat, consistent with the physiological significance of the enzyme in fatty acid metabolism.

Activation of long-chain fatty acids catalyzed by the long-chain acyl-coenzyme A synthetase (EC 6.2.1.3) is the first reaction in their metabolism. The long-chain acyl-CoA synthetase, originally described by Kornberg and Pricer (1), plays a key role in both synthesis of cellular lipids and degradation of fatty acids via β-oxidation.

In rat liver, long-chain acyl-CoA synthetase is localized in microsome (1), outer mitochondrial membrane (2, 3), and peroxisomal membrane (4, 5). Purified long-chain acyl-CoA synthetase from microsomes, mitochondria (6), and the peroxisomal fraction (7) of rat liver were identical with respect to catalytic properties, having the same molecular weights (approximately 76,000), amino acid composition, substrate specificities, and kinetic properties (6, 7). Identity of the enzymes in microsomes, mitochondria, and peroxisomal fraction of rat liver was also confirmed immunologically (8). These data strongly suggest that the same long-chain acyl-CoA synthetase is localized in microsomes, mitochondria, and peroxisomes. To our knowledge, this is the only enzyme that is distributed in three different organelles.

Regulation of long-chain acyl-CoA synthetase is important for overall fatty acid metabolism because this enzyme catalyzes the initial reaction in the metabolism of fatty acids. Numerous studies have measured the effects of different nutritional states on the activity of the long-chain acyl-CoA synthetase, but the results are not definitive, apparently because the enzyme in crude membrane preparations is not stable (9-14).

To analyze the molecular mechanism of regulation of the enzyme and the mechanism of targeting of the enzyme to different subcellular organelles, we isolated near full-length cDNAs encoding rat liver long-chain acyl-CoA synthetase. We describe here the primary structure of rat long-chain acyl-CoA synthetase deduced from the nucleotide sequence of a near full-length cDNA and discuss its functional domain. We provide the evidence for stimulation of the enzyme in rat liver by diet at the pretranslational level.

EXPERIMENTAL PROCEDURES

Materials—All enzymes were purchased from Takara Shuzou, Inc. (Kyoto, Japan) except as noted otherwise. 32P- and 3S-labeled nucleotides were products of either Amersham Corp. or Du Pont-New England Nuclear. Oligonucleotides were synthesized by the phosphoramidite method on an Applied Biosystems 381A oligonucleotide synthesizer.

General Methods—Preparation of plasmid DNA and poly(A)* RNA, restriction enzyme digestions, screening of cDNA libraries, agarose gel electrophoresis, and DNA blotting and hybridization were performed by standard procedures (15). Northern blot hybridization was carried out on Zeta probe membranes (Bio-Rad) (16). DNA probes were labeled with 32P by random hexanucleotide priming (17). To sequence the entire cDNA insert, the cDNA insert was shortened successively by exonuclease III and subcloned into pUC vectors. Nucleotide sequence was determined by the dideoxy-chain termination method (18) with specific oligonucleotide primers and T7 DNA polymerase (Sequenase*, United States Biochemical Corp., Cleveland). DNA sequence analysis was done on a NEC PC-8801RA5 with programs obtained from either Software Development Co. (Tokyo, Japan) or SciSoft Corp.

cDNA Cloning—cDNA libraries were constructed from rat liver poly(A)* RNA (19). Long-chain acyl-CoA synthetase cDNAs were detected with 32P-labeled oligonucleotides.

Peptide Sequence Analysis—Long-chain acyl-CoA synthetase was purified from rat liver microsomes (6). Peptides of the homogenous enzyme were prepared by digestion with lysyl endopeptidase (500:1) in 50 mM Tris (pH 9.0) and 5 M urea at 37 °C for 6 h. The peptides were fractionated by high pressure liquid chromatography using a Baker bond C8 0.46 x 25 cm reverse-phase column and a 0-60% gradient of acetonitrile in 0.1% trifluoroacetic acid; detection was at 215 nm. Each of the separated peptides was subjected to automated Edman degradation on a model 470A gas phase sequenator with on-line phenylthiohydantoin acid analysis (model 120A) (20).

Experimental Animals—Male Wistar strain rats (six/group, caged together) weighing 200-300 g were used in all experiments. Control rats were fed on the stock diet. Fasted rats were deprived of food for 48 h. Fed rats were fasted for 48 h followed by tree access to a high fat diet (20% soybean oil, 40% sucrose, 20% casein, 4% mineral mixture, 2% vitamin mixture, and 5% cellulose powder) or a fat-free diet (20% corn oil, 40% sucrose, 20% casein, 4% mineral mixture, 2% vitamin mixture, and 5% cellulose powder) for 8681
RESULTS AND DISCUSSION

Isolation of cDNA—Long-chain acyl-CoA synthetase was purified from rat liver microsomes (6). Purified enzyme was digested with lysyl endopeptidase, and the resulting fragments were fractionated by reverse-phase high pressure liquid chromatography (Fig. 1A). The amino acid sequences of five peptides were determined by automated Edman degradation (Fig. 1B). Attempts to determine the NH2-terminal sequence without lysyl endopeptidase digestion were unsuccessful. Based on the partial amino acid sequences, we synthesized three different oligonucleotide probes and used them to screen for the long-chain acyl-CoA synthetase cDNAs (Fig. 1C). We isolated poly(A)+ mRNA from normal and peroxisome-induced (22) rat liver and prepared two sets of cDNA libraries (19). To enrich the libraries for long cDNAs, we linearized all DNAs in each library, size-fractionated the plasmid DNAs on agarose gels, reoriented the larger plasmid DNAs, and used them to transform Escherichia coli. Probe A detected 11 clones from among 2 x 10^5 clones in the peroxisome-induced rat liver cDNA library. We also screened 1 x 10^5 clones from the size-fractionated normal rat liver library and isolated five positive clones. All of these clones were hybridized to probes B and C. The longest clone (pRACS15) from the peroxisome-induced rat liver cDNA library was subjected to restriction endonuclease mapping (Fig. 2), and its nucleotide sequence was determined.

Amino Acid Sequence of Rat Long-chain Acyl-CoA Synthetase—Fig. 3 shows the 3644-nucleotide sequence encoding rat long-chain acyl-CoA synthetase. We assigned the first ATG (nucleotide position 1-3) as the translational initiation codon because the nucleotide sequence flanking this putative initiation codon agrees with Kozak's consensus sequence (23). This codon initiates an open reading frame of 2097 base pairs, corresponding to 699 amino acids of M, 78,177, a value in close agreement with that determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified enzyme. All five lysyl endopeptidase peptides are found in the amino acid sequence deduced from the nucleotide sequence. The amino acid composition predicted by nucleotide sequence agrees with those determined by amino acid analysis of the purified enzymes (Table I). The NH2 terminus lacks a classical targeting sequence for endoplasmic reticulum or mitochondria. In vitro, the initial translation product has the same molecular weight as the purified enzymes, indicating that the primary structure lacks a signal sequence (8).

Our initial aim was to determine if long-chain acyl-CoA synthetases in the three different organelles had the same amino acid sequence. To examine this possibility, we sequenced both NH2- and COOH-terminal regions of two independently isolated near full-length cDNA clones from the normal rat cDNA library and four from the peroxisome-induced cDNA library. No differences in the amino acid sequence were found. We also analyzed the six near full-length cDNA clones by restriction enzymes. BamHI, EcoRI, HapII, HindIII, PstI, and XbaI; there were no differences within the coding region (data not shown). Long-chain acyl-CoA synthetases in three different organelles may have the same amino acid sequences.

Sequence Similarity between Rat Long-chain Acyl-CoA Synthetase and Firefly Luciferase—The amino acid sequence of rat long-chain acyl-CoA synthetase deduced from the nucleotide sequence was used to search the protein sequence data base of the National Biochemical Research Foundation (24). A rapid search routine (25) revealed a striking sequence similarity between rat long-chain acyl-CoA synthetase and firefly luciferase (26) (Fig. 4). The degree of sequence identity between long-chain acyl-CoA synthetase and firefly luciferase is 35.8%; a gap was counted as one substitution. When conservative replacements are included in the calculation, the similarity of long-chain acyl-CoA synthetase to firefly luciferase is 55.2%. This degree of similarity is highly significant and indicates that long-chain acyl-CoA synthetase is definitively related to firefly luciferase.

Firefly luciferase catalyzes the formation of oxyluciferin, AMP, CO2, and light from luciferin, O2, and ATP. The reac-

![Graph 1](image1)

**Fig. 1.** Separation of lysyl endopeptidase-digested peptides from the long-chain acyl-CoA synthetase: partial amino acid sequence and oligonucleotide probes. The long-chain acyl-CoA synthetase, purified from rat liver microsomes, was digested with lysyl endopeptidase. Resulting peptides were separated on a reverse-phase high pressure liquid chromatography (HPLC) (A). The deconvoluted peaks, A-E, represent peptides used for automated Edman degradation. Corresponding amino acid sequences are shown in B. According to these sequences, three oligonucleotide probes were synthesized. Sequences of oligonucleotides are depicted in C.

![Graph 2](image2)

**Fig. 2.** Rat long chain acyl-CoA synthetase cDNA. Restriction endonuclease map and sequencing strategy of the long-chain acyl-CoA synthetase cDNA are shown. The solid section of the cDNA indicates a coding region. Arrows indicate the extent and direction of sequencing reactions.
FIG. 3. Nucleotide sequence of the cDNA corresponding to rat long-chain acyl-CoA synthetase mRNA and the predicted amino acid sequence of the protein. Underlines indicate amino acid residues determined by automated Edman degradation. The sites to which N-linked carbohydrate could be attached (Asn-\(\text{X-Ser}\) or Asn-\(\text{X-Thr}\)) are indicated by asterisks.
Rat long-chain acyl-CoA synthetase catalyzed by firefly luciferase proceeds in two steps. The amino acid sequence of firefly luciferase is from Ref. 26.

Luciferase + luciferyl-AMP + PPi → luciferase + luciferin + ATP

The enzyme then catalyzes the formation of acyl-CoA and AMP from acyl-AMP and CoA. The two enzymes react with carboxyl groups of the two substrates and ATP to form adenylated intermediates in the first reactions and release AMP in the second reactions. Based on the similarity of the reactions catalyzed by the two enzymes, the amino acid sequence of the long-chain acyl-CoA synthetase, similar to the part of luciferase, may constitute a region where ATP and the carboxyl group of fatty acids interact to form acyl-AMP. The region of long-chain acyl-CoA synthetase similar to firefly luciferase is near the COOH terminus suggesting that the enzyme may project its COOH-terminal catalytic domain into the cytoplasm where fatty acid synthase and fatty acid binding protein are localized.

**Tissue Distribution and Regulation**—The tissue-specific pattern of expression of long-chain acyl-CoA synthetase mRNA was characterized by Northern blot hybridization analysis. Strong hybridization to a 3.8-kilobase mRNA was observed in liver, heart, and adipose tissue (Fig. 5), reflecting the importance of the long-chain acyl-CoA synthetase to fatty acid metabolism in these tissues. The level of expression in brain, lung, and small intestine was about 10% of that in liver, heart, and epididymal adipose tissue.

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**Fig. 5. Tissue distribution of the long-chain acyl-CoA synthetase mRNA in rat tissues.** Hybridization was performed with the 520-base pair EcoRV-HindIII fragment of the cDNA. Blots were prepared with total cellular RNA (15 µg) from small intestine (jejunum and ileum), lung, liver, heart, brain, and epididymal adipose tissue. The autoradiogram shown is a representation of six independent experiments which gave essentially identical results.
considered a constitutive enzyme because little change in enzyme activity was observed under various nutritional conditions (9-14). The current investigation reveals that the enzyme is stimulated at the pretranslational level in rat liver. Since long-chain acyl-CoA synthetase is an unstable membrane-bound enzyme, it may be impossible to evaluate the metabolic regulation of the enzyme by measuring its apparent enzyme activity in crude membrane preparations. Regulation of long-chain acyl-CoA synthetase is important, because the enzyme is the initial step for fatty acid metabolism. Our current result is consistent with the physiological significance of long-chain acyl-CoA synthetase in fatty acid metabolism.

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REFERENCES
Structure and regulation of rat long-chain acyl-CoA synthetase.
H Suzuki, Y Kawarabayasi, J Kondo, T Abe, K Nishikawa, S Kimura, T Hashimoto and T Yamamoto


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