Differentiation-induced Gene Expression in 3T3-L1 Preadipocytes

A SECOND DIFFERENTIALLY EXPRESSED GENE ENCODING STEAROYL-COA DESATURASE*

(Received for publication, May 12, 1989)

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Previously we isolated and characterized a differentially expressed gene from mouse 3T3-L1 preadipocytes that encodes stearoyl-CoA desaturase (SCD1; Ntambi, J. M., Buhrow, S. A., Kaestner, K. H., Chistry, R. J., Sibley, E., Kelly, T. J., Jr., and Lane, M. D. (1988) J. Biol. Chem. 263, 17291-17300). Genomic Southern blot analysis indicated the existence of another closely related gene. Here we report the isolation and characterization of this gene and the corresponding cDNA which encode a second stearoyl-CoA desaturase, SCD2, in 3T3-L1 adipocytes. SCD2 cDNA is 5 kilobase pairs in length and encodes a protein of 358 amino acids with >87% amino acid sequence identity to SCD1. RNase protection analysis reveals a 10-fold increase in the expression of SCD2 mRNA during 3T3-L1 preadipocyte differentiation. SCD2 mRNA is expressed constitutively at a high level in brain, is not expressed in liver, and its expression in kidney, adipose, and lung tissue is increased greatly by shifting mice from a diet containing unsaturated fatty acids to a diet devoid of fat. The tissue distribution and the dietary alteration of SCD1 mRNA expression differs markedly from that of SCD2 mRNA being absent from brain, constitutive in adipose tissue, and subject to negative control in liver by feeding a diet containing unsaturated fatty acids. The SCD2 gene spans approximately 15 kilobase pairs and consists of six exons and five introns, with intron/exon junctions similar to those of SCD1. As determined by primer extension analysis the start site of transcription maps 300 nucleotides upstream of the initiator methionine codon. Unlike the SCD1 gene, SCD2 lacks a typical "TATA" box in the 5'-flanking region, but has two "CCAT" boxes at positions -90 and -135 relative to the transcription initiation site. The SCD2 promoter contains a 140-base pair sequence (located between nucleotides -54 and -201) which possesses 77% sequence identity to a region (located between nucleotides -472 and -325) in the SCD1 promoter. There is a GC-rich sequence in the SCD2 promoter (at nucleotide -175) similar to the binding site for the nuclear transcription factor Sp1 as well as an element with homology to the core consensus sequence for the glucocorticoid regulatory element at position -500 and a potential CCAAT box/enhancer binding protein sequence at position -540. The SCD gene family provides a new model system for the study of differentiation-induced as well as tissue-specific metabolite controlled gene expression.

When appropriately induced, 3T3-L1 preadipocytes differentiate in culture into cells possessing the morphological and biochemical characteristics of adipocytes (1-3). Accompanying acquisition of the adipocyte phenotype is a dramatic rise in the cellular levels of lipogenic and lipolytic enzymes (3-8), as well as other proteins, e.g. the insulin receptor (9), that are expressed at high levels in adipocytes. Where investigated the increased expression of these proteins has been shown to result from an increased rate of transcription of their specific mRNAs (10) and a corresponding increase in their translation (11-13). Analysis of the regulatory elements of these genes should advance understanding of the factors that govern adipocyte differentiation.

Recently, we isolated the differentiation-induced gene encoding stearoyl-CoA desaturase 1 (SCD1,14) from 3T3-L1 preadipocytes. The function of this enzyme is the synthesis of unsaturated fatty acids as well as the regulation of this process. SCD1 catalyzes the Δ9-cis desaturation of fatty acyl-CoAs (15), the major products being palmitoleoyl- and oleoyl-CoA. Palmitoleic and oleic acid are the major (58% of total) storage form of unsaturated fatty acids in the triacylglycerols found in 3T3-L1 adipocytes (16). SCD activity is induced 20-100-fold during the differentiation of 3T3-L1 preadipocytes (16).

Genomic Southern analysis using SCD1 cDNA fragments as radioactive probes indicated the existence of related genes (14). In the present paper, we report the isolation and characterization of both the cDNA and the gene for a second differentially expressed mouse stearoyl-CoA desaturase (SCD2). The availability of these two genes should facilitate studies on the basis of their distinct tissue-specific expression as well as their regulation during preadipocyte differentiation.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and other nucleic acid modifying enzymes were obtained from Boehringer Mannheim, Bethesda Research Laboratories, Pharmacia P-L Biochemicals, New England Biolabs, and United States Biochemical Corp. Radionucleotides were obtained from Du Pont-New England Nuclear. Nylon membranes were from Amersham Corp.

Cell Culture—Cell culture and differentiation of 3T3-L1 preadipocytes were performed as described previously (14).

The abbreviations used are: SCD, stearoyl-CoA desaturase; bp, base pairs; kb, kilobase pairs; C/EBP, CCAAT box/enhancer binding protein.

*This work was supported in part by Research Grant NIDDK 38418 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Fig. 1. Map of the mouse 3T3-L1 SCD2 gene. The top scale indicates the nucleotide position in kilobases. The two overlapping genomic clones (AS18 and AE14) which encompass the entire gene are shown below the scale. Also shown is a restriction map of the gene. The solid and open boxes indicate the coding and the 5' and 3' noncoding exon sequences, respectively. Two overlapping cDNA clones (λ32 and λ33) which represent the entire mRNA are illustrated below. Restriction enzyme sites: B, BamHI; E, EcoRI.
in Fig. 3. The amino acid substitutions are concentrated in the amino-terminal one-third of the protein. All three SCD sequences exhibit virtually superimposable hydrophoby plots (data not shown).

Cloning of the SCD2 Gene—In order to obtain the complete gene for SCD2, we used a 0.5-kb intron fragment of ΐΔE14 to screen a 3T3-L1 genomic library (14). The two clones isolated were found to be identical and designated AS18 (Fig. 1). The amino acid substitutions are concentrated in the amino-terminal one-third of the protein. All three SCD sequences exhibit virtually superimposable hydrophoby plots (data not shown).
The nucleotide sequences of the exon-intron junctions were determined as described under “Experimental Procedures.” The exon-intron junctions were positioned by comparing the nucleotide sequence of the genomic DNA with that of the cDNA. The GT and AG dinucleotides of the introns which correspond to the splice consensus sequences are underlined. The sizes of the exons were determined by sequencing. All intron/exon boundaries occur at the same sites as in the SCD1 sequence (25). Only amino acids diverging from the SCDZ sequence are shown for SCD1 and rat SCD. Overall, the SCDZ and SCD1 sequences are 87% identical; the SCDS and rat SCD sequences are 86% identical.

The transcription initiation site for SCD2 was mapped by primer extension analysis (Fig. 5) using poly(A)+ RNA from fully differentiated 3T3-L1 adipocytes. The strongest band corresponds to base 1 in the cDNA sequence (Fig. 2). A similar primer extension analysis revealed that the same initiation site is used in brain (data not shown). A SCD2 primer extension product could not be detected in mRNA isolated from 3T3-L1 preadipocytes suggesting that expression of SCD2 mRNA is induced upon differentiation into adipocytes (see below). The 5'-untranslated leader of SCD2 markedly diverges in sequence and length from that of SCD1 (14), the former being 120 nucleotides longer than the latter.

Differential Expression of SCD2 mRNA—To determine whether SCD2, like SCD1, is induced during differentiation of 3T3-L1 preadipocytes into adipocytes, we isolated total RNA every 24 h during the course of differentiation. Message levels for SCD1 and SCD2 were analyzed by RNase protection using the divergent untranslated 5' or 3' regions of each cDNA as probes. As shown in Fig. 6, both expression of SCD1 and SCD2 mRNAs are induced during differentiation. Confirming previous observations (14), SCD1 mRNA is expressed on day 3 (following induction of differentiation), peaks on day 5, and remains at high levels through day 7. SCD2 RNA is detectable on day 2, peaks on day 4, and is not as abundant as SCD1 mRNA. At day 5, SCD1 mRNA is approximately 5 times as abundant as SCD2 mRNA.

It has been well documented that hepatic stearoyl-CoA desaturase levels in rats can be increased by a shift from a natural diet containing unsaturated triacylglycerides to a regimen of fasting followed by refeeding a fat-free diet (23), conditions under which the animal is required to synthesize...
all monounsaturated fatty acids. We have demonstrated previously that hepatic SCD1 mRNA levels in mice are induced by a similar feeding protocol (14). To assess the tissue distribution of SCD2 mRNA and to determine whether SCD2 is induced by dietary conditions as well, 3- to 4-week-old mice were starved and then refed a fat-free diet (see “Experimental Procedures”) or fed ad libitum. Total RNA from seven tissues was isolated and SCD1 and SCD2 mRNA levels were measured by the RNase protection method (Fig. 7). As observed previously (14) SCD1 is expressed constitutively (Fig. 7B) in adipose tissue, induced substantially in liver and to a lesser extent in kidney and lung, and is absent in brain, heart, and spleen under either dietary condition. On the other hand, SCD2 mRNA (Fig. 7A) is constitutively expressed in brain, is induced by refeeding a fat-free diet in adipose, kidney, and lung and is present at low levels in heart and spleen, but is not detected in liver from both groups of mice.

The 5'-Flanking Sequence of the SCD2 Gene—Part of the 5'-flanking region of the SCD2 gene was sequenced by the dyeoxy chain termination method after subcloning the appropriate fragments into pGEM plasmids. The promoter region (Fig. 8) contains a weak "TATA" nucleotide sequence homology at position -25 relative to the major start site of transcription. Two CCAAT boxes are found at positions -90 and -135, and a potential Sp1 binding site is located at position -175. Further upstream, at nucleotide -500, is a nucleotide sequence similar to core glucocorticoid regulatory elements, and at position -540 is a sequence closely related to the suggested consensus sequence for binding of the nuclear transcription factor C/EBP (26).

Of particular interest is the fact that 146 bp of promoter sequence (nucleotide -201 to -54) in the SCD2 gene possesses

77% nucleotide identity to the promoter sequence (nucleotide -472 to -325) in the SCD1 gene (see Fig. 9). The conserved sequence includes two CCAAT boxes which are located, only in the SCD2 gene, at the typical position in proximity to the start site of transcription. The Sp1 consensus binding site (GC-box) at position -175 in the SCD2 gene is not found at the corresponding location (-450) in the conserved segment of the SCD1 promoter. The SCD1 promoter does, however, possess a GC-box at nucleotide -210.

DISCUSSION

Stearoyl-CoA desaturase is the key regulatory enzyme of unsaturated fatty acid biosynthesis, the major form of fatty acid stored in the triacylglycerols of mouse adipocytes. It was shown previously that the activity of this enzyme increases dramatically during differentiation of 3T3-L1 preadipocytes into adipocytes (16). More recently, we isolated the gene for a stearoyl-CoA desaturase (SCD1) from 3T3-L1 preadipocytes. Here we describe the isolation and characterization of a previously unrecognized closely related gene, SCD2, which encodes an isoform of this differentially expressed enzyme.

The SCD2 gene spans about 15 kb and has six exons and five introns. The cDNA is a 5 kb in length and contains a single open reading frame of 358 amino acids, followed by an unusually long 3658-bp 3' untranslated sequence. This arrangement is reminiscent of that for SCD1 (14). In contrast to other genes with large 3' untranslated regions that often contain multiple polyadenylation signals allowing for the synthesis of mRNAs of varying length, the SCD2 gene has only one such sequence (AAUAAA) 24 bases upstream from the poly(A) track. The functional role of the large 3' untranslated region is unknown. From genomic Southern blots both in this (Fig. 4) and a previous paper (14), it appears that the mouse SCD gene family may contain a third closely related
gene, as the SCD1 and SCD2 genes do not account for all bands detected in high stringency genomic Southern blots.

Comparison of the three SCD amino acid sequences reported thus far (14, 25) and that in this report shows a highly conserved carboxyl-terminal half of the enzyme molecule, suggesting that this region may contain the catalytic site. This is supported by a recent report in which a deletion of the first 26 amino acids did not affect the catalytic activity of the SCD protein. A potential glucocorticoid regulatory core element and a potential C/EBP binding site, which exhibits sequence homology, is located 201 base pairs upstream from the SCD1 mRNA start site.

In mice fed a normal diet containing unsaturated fatty acids, SCD2 mRNA is found at high levels only in brain. This is supported by a recent report in which a deletion of the first 26 amino acids did not affect the catalytic activity of the SCD protein. A potential glucocorticoid regulatory core element and a potential C/EBP binding site, which exhibits sequence homology, is located 201 base pairs upstream from the SCD1 mRNA start site.

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**Acknowledgments**—We thank Dr. Robert J. Christy, Eric Sibley, and Dr. Vincent Yang for helpful discussions and Natalie Tumminia.
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