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EXPRESSION OF ONLY ONE OF TWO TYPES OF mRNA TRANSCRIBED FROM THE SERINE DEHYDRATASE GENE IS REPRESSED IN HEPATOMA CELLS

Koichi Matsuda, Chiseko Noda, and Akira Ichihara

Institute for Enzyme Research, University of Tokushima,
Tokushima 770, Japan

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Summary: The expressions of mRNA for serine dehydratase in H4IIE, HTC, and HepG2 hepatoma cells were investigated. Of the two types of mRNA transcribed from the single copy of the rat serine dehydratase gene, expression of only that encoding 35 kDa serine dehydratase is repressed in these cells. The other type coding the 8.9 kDa truncated translation product is expressed at a similar level to that in hepatocytes in primary culture and is not under hormonal control.

Serine dehydratase(SDH, EC4.2.1.13), which catalyzes formation of pyruvate from L-serine, is mainly located in the liver. Transcription of the gene encoding rat SDH in the liver is switched on after birth and then increases gradually to the adult level (1-4). However, when adult rats are partially hepatectomized (4) or treated with a chemical carcinogen (5), SDH activity decreases markedly, indicating that when mature hepatocytes enter the proliferating state, their expression of SDH is repressed. Previous studies have demonstrated that the enzyme activity in Reuber H35 and Novikoff hepatoma is negligible (6,7). However, the SDH activity in Morris 5123 and 7793 hepatomas is extremely high (7) and this high activity has been thought to be due to stabilization of the mRNA for SDH (8). Therefore, it seems of interest to study the expression of SDH mRNA in hepatoma cells.

Recently, we (9,10) and Ogawa *et al.* (11,12) cloned cDNAs encoding rat SDH and the rat SDH gene. These studies demonstrated that two types of mRNA are transcribed from a

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single copy of the SDH gene per haploid genome by use of different transcription initiation sites and alternative splicing. SDH mRNA I encodes a 35 kDa protein that corresponds with the purified enzyme, while SDH mRNA II has a termination codon in the fifth exon resulting in a truncated product of 8.9 kDa, which is not thought to have any enzyme activity and whose biological function is unknown. Ogawa *et al.* reported that in liver the ratio of the amounts of type I and II mRNAs is approximately 20:1 (12).

We investigated the expressions of SDH in H4IIE, HTC and HepG2 cells, which retain some liver-specific functions. As shown in this paper, we found that the hepatoma cells maintain a similar level of SDH mRNA II to that in adult rat hepatocytes in primary culture and that their loss of SDH activity is due to decreased expression of SDH mRNA I, whereas a significant level of SDH mRNA I is present in cultured hepatocytes.

Materials and Methods

Cell Culture: Hepatocytes were isolated from male rats of the Wistar strain, seeded at a density of 1×10^5 cells/cm^2, and cultured as described previously (13). After an attachment period of 2 h, the medium was replaced by fresh medium and incubation was continued for 24 h in the presence of 5% calf serum, but the absence of hormones. Then the cultures were refed with fresh medium, supplemented with 10^{-7} M dexamethasone plus 5×10^{-5} M dibutyryl cyclic AMP(bt_2cAMP) when indicated, and were incubated for further 24 h before harvesting.

H4IIE (6), HTC (14), and HepG2 (15,16) cells, derived from rat and human hepatoma cells were routinely cultured on plastic culture dishes at 37°C in an atmosphere of 95% air and 5% CO_2 in Dulbecco's modified Eagle's medium(DMEM) supplemented with 10% fetal bovine serum. Cultures were refed every 24 h and the cells growing to subconfluence were incubated with or without 10^{-7} M dexamethasone and 5×10^{-5} M bt_2cAMP for the last 24 h.

Assay of Enzyme Activities: Enzyme activities of SDH and tyrosine aminotransferase were assayed as described previously (13).

Northern Blot Analysis: Total RNA was isolated from cells and rat liver by the guanidine isothiocyanate procedure (17). Samples of 10μg of RNA were denatured with formamide and transferred to nylon filters after electrophoresis. Hybridization to an SDH cDNA fragment (9) labeled by random priming with Klenow polymerase was carried out as described (17).

Primer Extension Analysis: Two 24-mer oligonucleotides, 5' CCCAATTGCTGGGAT-CACAGGTAT 3' (Primer 1) and 5' CATGGGTTCCACGGCAGAAGTCTC 3' (Primer 2), complementary to the sequences of the 5' regions of SDH mRNA I and II and of SDH mRNA II, respectively, were synthesized in a DNA synthesizer (Applied Biosystem Inc.).

The oligonucleotides were end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP. The labeled nucleotides were annealed to total RNA, which was prepared using Oligotex dT-30 (Takara Shuzo), and extension was carried out using AMV reverse transcriptase (Life Sciences Inc.)(17). The product was analyzed on a sequencing gel.

Immunoprecipitation: Cells were labeled with 50 μCi/ml of [^{35}S] methionine for 7 h in methionine-free media. Preparation of cell lysates and immunoprecipitation were carried out as described previously (18).

Results and Discussion

There are many reports of studies using H4IIE, HTC and HepG2 cells on the mechanisms regulating the expressions of the liver specific genes phosphoenolpyruvate carboxykinase, tyrosine aminotransferase and albumin (6,14,16,19,20). However, as shown in Table 1, the SDH activity in these hepatoma cells is negligible, even in the presence of dexamethasone and bt_2cAMP which cause several fold induction of SDH in adult rat hepatocytes in primary culture as described previously (13,18,21). The loss of SDH activity in these hepatoma cells is in contrast with their significant expression of tyrosine aminotransferase.

Northern blot analysis was performed with total RNA extracted from rat liver, cultured hepatocytes, H4IIE, HTC and HepG2 cells as shown in Fig. 1. SDH mRNA (approximate 1.5 Kb) was detected only in the liver and cultured hepatocytes treated with

Table 1. Activities of serine dehydratase and tyrosine aminotransferase in cultured hepatocytes and hepatoma cell lines

Cells	Inducers	Activity (mU/mg protein)	
		SDH	Tyrosine aminotransferase
Cultured hepatocytes	-	5.6	10.4
	+	34.7	270
H4IIE	-	NG	3.84
	+	NG	70.8
HTC	-	NG	5.48
	+	NG	31.9
HepG2	-	NG	0.79
	+	NG	1.05

NG, negligible value.
Inducers, 10^{-7} M dexamethasone and 5 x 10^{-5} M bt_2cAMP were added when indicated. Results are means for 3 experiments.

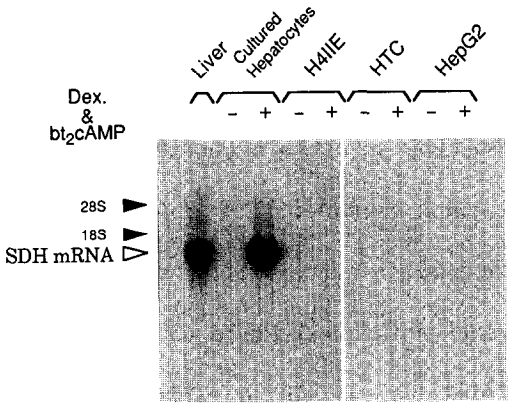


Fig. 1. Northern blot hybridization analysis of SDH mRNA of rat liver, hepatocytes in primary culture, and hepatoma cells. Total RNA was probed with a 850 bp *Pst*I fragment of the SDH cDNA. The cells were treated with 10^{-7} M dexamethasone and 5 x 10^{-5} M bt_2cAMP to induce expression of SDH for 24 h when indicated.

dexamethasone and bt_2cAMP. No SDH mRNA was detected in hepatocytes cultured in the absence of the inducers, because expression of SDH was markedly decreased in this condition (21). As described previously, two types of SDH mRNA are transcribed from a single copy of SDH gene (Fig. 2). These two types of SDH mRNA cannot be distinguished

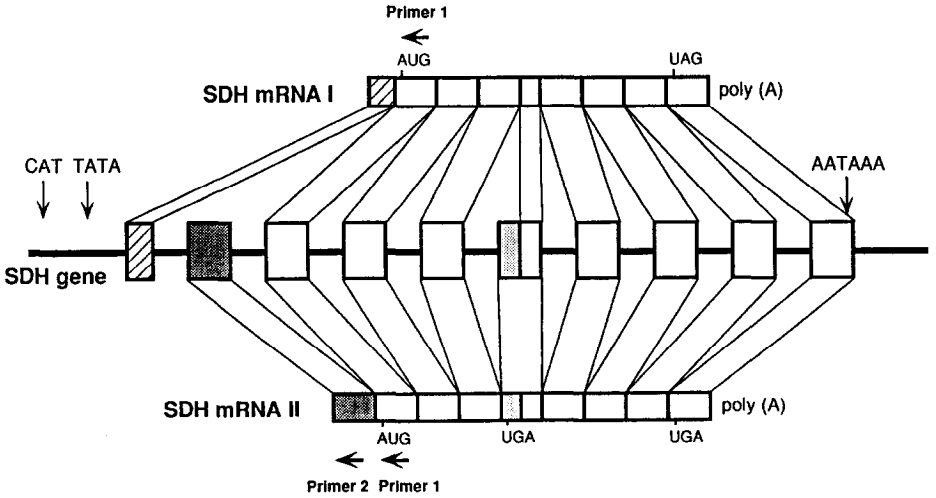
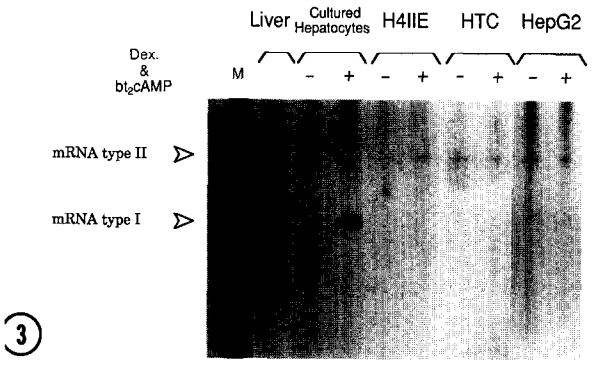


Fig. 2. Scheme of rat SDH gene transcription. mRNA I and II are transcribed alternatively from different initiation sites of the SDH gene. Then each mRNA primary transcript is spliced in a different way to become mature mRNA. The corresponding sites for the primers used in primer extension analysis are indicated.

by Northern blot analysis, because they are approximately equal in nucleotide length and differ only in their sequences encoded by exon 1 and part of exon 5.

Although SDH mRNA was not detected in the hepatoma cells by Northern blot analysis, to determine which type(s) of SDH mRNA expression is repressed in the hepatoma cells, we carried out primer extension with two different oligonucleotides as primers. The sequences from initiation codon to -77 are same between SDH mRNA I and II, but those of further upstream are different between them. Primer 1 and Primer 2 correspond to position -64 to -41 of both SDH mRNAs and position -149 to -126 of SDH mRNA II, respectively. Thus, in primer extension analysis using Primer 1, the expected sizes of the products of SDH mRNA I and II are 107 and 181 nucleotides, respectively. On the other hand, a product of 96 nucleotides would be expected using Primer 2. As shown in Fig. 3, SDH mRNA I probed by Primer 1 was present only in cultured hepatocytes treated with dexamethasone and bt_2cAMP. In contrast, SDH mRNA II was expressed not only in cultured hepatocytes, but also in the hepatoma cells, and its expression was not influenced by the hormones. Analysis by Primer 2 showed expression of SDH mRNA II in all cells examined (data not shown). The reason why expression of SDH mRNA II was not detectable in the hepatoma cells and hepatocytes on Northern blot analysis is thought to be that its levels were very low in these cells.

To identify the translated products of the two SDH mRNAs, cells were labeled with [^{35}S]methionine, the cell extracts were subjected to immunoprecipitation with anti-SDH polyclonal antibody, and the immunoprecipitates were analyzed by SDS-PAGE. The product of mRNA I (35 kDa) was found only in cultured hepatocytes treated with dexamethasone and bt_2cAMP(Fig. 4), consistent with the results of primer extension analysis. However, 8.9 kDa peptide, the translation product of SDH mRNA II, was not detectable in any cells examined including cultured hepatocytes. The failure to demonstrate the 8.9 kDa peptide may have been due either to its low level or to a weak reaction of the polyclonal antibody with this peptide. The various bands with molecular



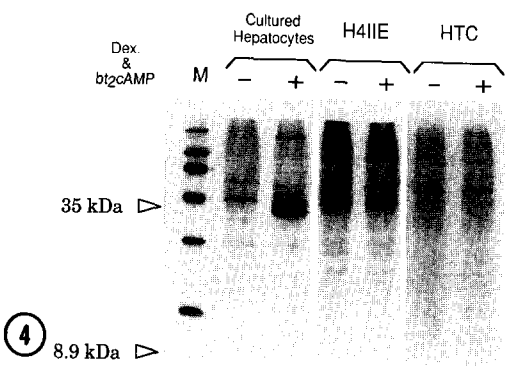


Fig. 3. Primer extension analysis with Primer 1. Total RNAs from the cells were primed with ^{32}P-labeled Primer 1. The nucleotide sequence of the primer was as described under "Materials and Methods". Hormone treatments were as described in the legend for Fig. 1.

Fig. 4. Immunoprecipitation of SDH from hepatocytes in primary cultures, H4IIE and HTC cells. Cells were plated in 90-mm dishes. The cells were labeled with 50 μCi/ml of [^{35}S]methionine in methionine-free medium for 7 h. When indicated, the cells were treated with dexamethasone and bt_2cAMP for 24 h before harvesting. Immunoprecipitation was carried out using polyclonal anti-SDH antibody as described.

weights of more than 35 kDa detected seemed to be nonspecific. From these results we conclude that decreased expression of SDH mRNA I, but not that of SDH mRNA II, in hepatoma cells results in loss of SDH enzyme activity.

Previously, we demonstrated that the promoter region of the SDH gene is located within 765 bp upstream from the transcription initiation site of SDH mRNA I (22). There are no typical TATA and CAT boxes, but instead there are AATAAA and CATT sequences in this region (10,11). In addition to these sequences, several GC box sequences are located upstream of the transcription initiation site. SDH mRNA II is transcribed 172 bp downstream from the cap site of mRNA I. Since promoter like sequences, such as TATA, CAT, and GC boxes, are not found in the proximal sequence upstream of the cap site of mRNA II, the promoter region for transcribing mRNA II may be partly shared with that for mRNA I transcription.

We did not investigate the mRNA level in cell lines derived from Morris 5123 and 7793 hepatomas, in which SDH activity is extremely high. The results described in this paper

demonstrated that SDH mRNA II is constitutively expressed even in hepatoma cells, while SDH mRNA I is expressed specifically in differentiated hepatocytes. When the 5' flanking sequence of the SDH gene was fused with chloramphenicol acetyltransferase and this fused gene was transfected into H4IIE and HTC cells, no promoter activity of the SDH gene was detectable, whereas the promoter was active in cultured hepatocytes (22). This means that quantitative or qualitative change of a transcription factor(s) occurs in these cell lines. Studies on how the level of this factor(s) is regulated in hepatocytes and hepatoma cells may provide a clue for understanding the molecular mechanisms of liver-cell differentiation and dedifferentiation.

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