

Primary structure of rat liver serine dehydratase deduced from the cDNA sequence

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The nucleotide sequence of serine dehydratase mRNA of rat liver has been determined from a recombinant cDNA clone, previously cloned in this laboratory, and from a recombinant cDNA clone screened from a primer-extended cDNA library. The sequence of 1322 nucleotides includes the entire protein coding region and noncoding regions on the 3'- and 5'-sides. The deduced polypeptide consists of 327 amino acid residues with a calculated molecular mass of 34462 Da. Comparison of the amino acid sequences of the serine dehydratase polypeptide with those of biosynthetic threonine dehydratase of yeast and biodegradative threonine dehydratase of *E. coli* revealed various extents of homology. A heptapeptide sequence, Gly-Ser-Phe-Lys-Ile-Arg-Gly, which is the pyridoxal-binding site in the yeast and *E. coli* threonine dehydratases, was found as a highly conserved sequence.

Serine dehydratase; cDNA cloning; Nucleotide sequence; Pyridoxal phosphate-binding site; (Rat liver)

1. INTRODUCTION

Serine dehydratase (SDH) catalyzes the pyridoxal phosphate-dependent dehydration of serine to ammonia and pyruvate. The enzyme in rat liver is composed of two identical subunits of approximately 34 kDa [1,2], and there are 2 mol of pyridoxal phosphate per dimer [1]. Expression of this enzyme is known to be liver-specific and to contribute to gluconeogenesis from serine, although L-serine-pyruvate aminotransferase also catabolizes serine to pyruvate. The extents of contribution of the two pathways to serine catabolism differ according to the species and the physiological state [3]. SDH is highly induced in some physiological conditions, such as diabetes or

intake of a high protein diet. To investigate hormonal regulation of this enzyme in vitro, we used primary cultures of rat hepatocytes and demonstrated that both glucocorticoids and glucagon (or cAMP) were required to induce translatable mRNA of this enzyme [4]. The hormone-requirement for induction of this enzyme is different from that of tryptophan 2,3-dioxygenase, which is also a liver-specific enzyme, transcription of the gene of the latter enzyme being markedly stimulated by glucocorticoids alone [5].

Previously, DNA of about 1140 nucleotides long that was complementary to SDH mRNA from rat liver was cloned in this laboratory [6]. Nuclear run-off experiments with this cDNA as a probe showed that glucocorticoids and glucagon act as co-inducers to stimulate the transcription of the SDH gene (Noda, C. et al. submitted). To study the molecular mechanism of hormonal regulation of SDH, the gene must be isolated and the *cis*-acting sequence(s) and *trans*-acting factor(s) must be identified. Recently, we have cloned the rat SDH gene (unpublished) and are determining its DNA sequence. In this report we present the complete amino acid sequence of SDH deduced from the

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cDNA sequence, compare it to the amino acid sequences of yeast and *E. coli* threonine dehydratases, and predict the sequence of the active site that binds to pyridoxal phosphate.

2. MATERIALS AND METHODS

2.1. Materials

The enzymes used in primer extension experiments were as described previously [6]. Restriction endonucleases were obtained from Toyobo (Osaka) and Takara Shuzo (Kyoto). T₄ polynucleotide kinase and T₄ DNA ligase were purchased from Takara Shuzo and New England Bio Laboratory, respectively. A dideoxy sequencing kit and pUC18 and 19 vectors were obtained from Takara Shuzo; [α -³²P]CTP and [γ -³²P]ATP were from New England Nuclear.

2.2. SDH cDNA clone and primer extension

The construction of plasmid pSDH4 which comprises a cDNA sequence from rat SDH of about 1140 bases has been published [6]. In obtaining the remaining 5'-sequence of the complete cDNA sequence by primer extension, an oligonucleotide of 24 bases 5'-CACTTTGGACAATGCCATGCTGTC-3' was synthesized. This oligonucleotide was kindly synthesized by members of Otsuka Pharmaceutical Research Laboratories (Tokushima). This oligonucleotide is complementary to the SDH mRNA strand and its sequence is located between nucleotide 35 and 58 from the 5'-end of the cDNA insert of pSDH4. Using this oligonucleotide a primer-extended cDNA library was constructed from rat liver poly(A) RNA in pBR322 by a slight modification of the standard method [7]. *E. coli* DH 1 cells were transformed with the recombinant DNAs and the transformants obtained were screened with the 5'-end labeled oligonucleotide of 24 bases, which has the same sequence as that used in the primer extension, as a probe.

2.3. Nucleotide sequence analysis

Restriction fragments were isolated from low-melting-point agarose and ligated into plasmids pUC18 and 19 [8]. DNA sequencing was carried out by the dideoxy chain-termination method [9].

3: RESULTS AND DISCUSSION

Fig.1 shows the structure of SDH mRNA and the restriction map of the SDH cDNA inserts. One of the cDNA clones (pSDH4), which was previously obtained in this laboratory and reported to have an insert of about 1000 base pairs [6], comprises about 1140 base pairs of the cDNA sequence in the pBR322 vector. This clone covers more than 70% of the entire sequence of SDH mRNA, since the mRNA is about 1500 nucleotides long, as estimated by Northern blot analysis [6]. Nucleotide sequence analysis revealed that this cDNA clone

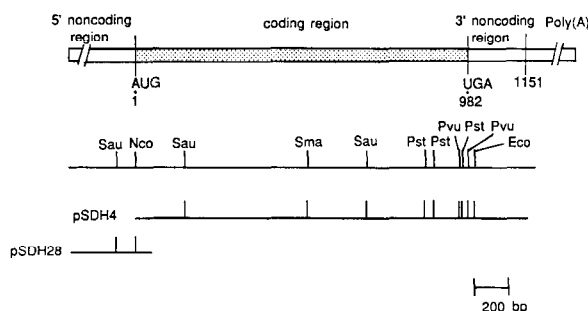


Fig.1. Restriction endonuclease cleavage sites of the SDH cDNA inserts and organization of SDH mRNA. The numbers below the structure of the mRNA indicate the length in nucleotides from AUG to termination codon UAG and to the poly(A) addition site. Sau, *Sau3A*; Nco, *NcoI*; Sma, *SmaI*; Pst, *PstI*; Pvu, *PvuII*; Eco, *EcoRV*. bp, base pairs.

did not contain the 5'-noncoding region of the SDH mRNA strand. To obtain the rest of the cDNA sequence, we constructed a primer extended cDNA library from rat liver poly(A) RNA by use of a synthetic oligonucleotide of 24 bases located in the 5'-position of the pSDH4 insert, as described in section 2. Of the 400 transformants screened 1 clone, designated as pSDH28, covered the 5'-noncoding region of the SDH mRNA strand.

The nucleotide sequence of these cDNAs and the primary structure of rat SDH deduced from the cDNA sequence are shown in fig.2. The sequence of about 1322 nucleotides includes the entire coding and 3'-noncoding regions and most, if not all, 5'-noncoding sequence. The 3'-noncoding region consists of 159 nucleotides, excluding the poly(A) tail. The polyadenylation signal (AATAAA), which is common to mRNAs [10,11], was located about 27 nucleotides upstream from the poly(A) addition site. The protein coding region is 981 nucleotides long, extending from ATG to the termination codon TGA. However, an alternative possibility is that the open reading frame is located at either ⁴⁹ATG⁵¹ or ⁹¹ATG⁹³. Although the N-terminal amino acid sequence of SDH is unknown, we suspect that the ATG located at nucleotides 1 to 3 is the initiation codon for the following two reasons. First, there is a termination codon TGA at nucleotides -36 to -34 in the 5'-noncoding region, and following this termination codon ¹ATG³ is the first initiation codon except ⁻⁵ATG⁻³. But, it is unlikely that ⁻⁵ATG⁻³ is the

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GAGACTTGAG GTAAACTATT GGACCCGAAC TCCACTCTCC AGGACACAGA CAAAATCTCC CTCTCGCTC GCTCTGTCCA ACTCCTCTCG -83

Met Ala 2
ATG GCT 6

TGCGGGGTGC GGCGGGGCAT ACCTGTGATC CCAGCAATTG GGAGACTGAG ACAGGAGGAT CCAACCTTCA AAGCTACATG CC 27

Ala Gln Glu Ser Leu His Val Lys Thr Pro Leu Arg Asp Ser *Met Ala Leu Ser Lys Val Ala Gly Thr Ser Val 27
GCC CAG GAG TCC CTG CAC GTG AAG ACC CCA CTA CGT GAC AGC ATG GCA TTG TCC AAA GTG GCC GGC ACT AGT GTG 81

Phe leu Lys Met Asp Ser Ser Gln Pro Ser Gly Ser Phe Lys Ile Arg Gly Ile Gly His Leu Cys Lys Met Lys 52
TTC CTT AAG ATG GAC AGC TCT CAG CCC TCT GGC TCC TTC AAG ATC CGA GGC ATT GGG CAT CTC TGC AAG ATG AAG 156

Ala Lys Gln Gly Cys Lys His Phe Val Cys Ser Ser Ala Gly Asn Ala Gly Met Ala Thr Ala Tyr Ala Ala Arg 77
GCA AAA CAA GGC TGT AAA CAT TTC GTC TGC TCT TCA GCG GGC AAC GCG GGC ATG GCG ACT GCC TAT GCT GCC AGG 231

Arg Leu Gly Leu Pro Ala Thr Ile Val Val Pro Ser Thr Thr Pro Ala Leu Thr Ile Glu Arg Leu Lys Asn Glu 102
AGG CTG GGC CTC CCA GCC ACT ATT GTT GTG CCA AGC ACC ACA OCT GCC CTC ACC ATT GAG CGG CTG AAG AAC GAA 306

Gly Ala Thr Val Glu Val Val Gly Glu Met Leu Asp Glu Ala Ile Gln Leu Ala Lys Ala Leu Glu Lys Asn Asn 127
GGG GCC ACA GTT GAA GTG GTG GGA GGC TCT GAT GAT GGC CAA CTG GCC AAG GCT CTG GAA AAG AAC AAC 381

Pro Gly Trp Val Tyr Ile Ser Pro Phe Asp Asp Pro Leu Ile Trp Glu Gly His Thr Ser Leu Val Lys Glu Leu 152
CCA GGT TGG GTG TAC ATC TCC CCC TTC GAT GAC OCT CTC ATC TGG GAA GGC CAC ACT TCC CTT GTG AAG GAG CTG 456

Lys Glu Thr Leu Ser Arg Ala Lys Pro Gly Ala Ile Val Leu Ser Val Gly Gly Gly Gly Thr Cys Gly Val Val 177
GAG GAG ACA CTG ACC GCC AAG CCC GGG GGC ATT GTG TCT TCT GGC GGT GGA GGC CTG CTG GGA GTG GTC 531

Glu Gly Leu Arg Glu Val Gly Trp Glu Asp Val Pro Ile Ile Ala Met Glu Thr Phe Gly Ala His Ser Phe His 202
CAG GGG CTG CCG GAG GTG GGC TGG GAG GAT GTG CCC ATC ATC GCC ATG GAG ACC TTC GGC GCC CAC AGC TTC CAC 606

Ala Ala Val Lys Lys Glu Gly Lys Leu Thr Leu Pro Lys Ile Thr Ser Val Ala Lys Ala Lys Val Asp Thr 227
GCT GCC GTC AAG GAA GGA AAG CTG GTC ACC CTG CCC AAG ATC ACC AGT GTT GCC AAG GCC TTG GGT GTG AAC ACT 681

Val Gly Ala Gln Thr Leu Lys Leu Phe Tyr Glu His Cys Ile Phe Ser Glu Val Ile Ser Asp Gln Glu Ala Val 252
GTG GGG GCA CAG ACC CTG AAG CTG TTT TAC GAA CAC CCC ATT TCT TCT TCA GAC CAG GAG GCT GTG 756

Thr Ala Ile Glu Lys Phe Val Asp Asp Glu Lys Ile Leu Val Glu Pro Ala Cys Gly Ala Ala Leu Ala Ala Val 277
ACT GCT ATC GAG AAG TTC GTA GAC GAT GAG AAG ATC CTG GTG GAG CCC GCG TGT GGC GCT GCC CTG GCT GCA GTG 831

Tyr Ser Gly Val Val Tyr Cys Arg Leu Gln Ala Glu Gly Arg Glu Thr Pro Leu Ala Ser Leu Val Ile Val 302
TAC AGC GGT GTG GTG TGC AGG CTG CAG GAT GCA GGC CCA CTG CAA ACC CCA CTG GCT TCG CTG GTT GAT ATT GTG 906

Cys Gly Gly Ser Asn Ile Ser Leu Ala Gln Leu Gln Ala Leu Lys Ala Gln Leu Gly Leu Asn Glu Leu Leu Lys 327
TGT GGT GGC AGC AAC ATC AGC CTG GCA CAG CTG CAG GCA CTC AAG GCA CAG CTG GGC CTG AAT GAG CTA CTC AAG 981

TGA TATCTGCTGC TGCCCTGGCC ACCCTGAGGG GTCACCAGCA CCCTGAGTA GGCTGGGTGG GGTCCGCCT GACAGTGCC CACCCTC 1071

CTT TATCCATGTT TATAATATGC ACTTTTTCAT TGTAATAAAA AATTAAATATA TATATGTTA TAAAGC AAAAAAAAAA 1150

Fig. 2. Nucleotide sequence of the SDH cDNA and the deduced amino acid sequence of SDH. The upper numbers on the right represent amino acid residues of the polypeptide from the N-terminus. Nucleotides in the SDH sequence are numbered from the translational initiation site (lower numbers). The predicted binding site for pyridoxal phosphate in the amino acid sequence is overlined. The polyadenylation signal is underlined. The asterisk indicates the i alternative open reading frame discussed in the text.

initiation codon because this would cause a frame shift and then a nonsense codon in the coding region. Second, in the 5'-noncoding regions of mRNAs, the sequence $^{-5}\text{CC}^{\text{A}}\text{CC}^{-1}$ just upstream from the initiation codon is reported to be conserved [12]. The sequence in SDH cDNA, $^{-5}\text{ATGCC}^{-1}$, partly matches this conserved sequence. If $^1\text{ATG}^3$ is the true initiation codon, the coding region encodes a protein of 327 amino acids. The deduced amino acid composition of this protein is consistent with that of the native protein reported by Inoue et al. [2]. From the amino acid sequence, the molecular mass of SDH was calculated to be 34462 Da, which is in good agreement with the approximate molecular mass of 34 kDa for the subunit estimated by SDS-polyacrylamide gel electrophoresis [2]. The pSDH4 clone contained sequences coding for amino acids 4–327 and the entire 3'-noncoding region including 10 bases of the poly(A) tail. The pSDH28

clone obtained by primer extension, contained sequences coding for amino acids 1–22 and 172 nucleotides in the 5'-noncoding region. However, this clone may not contain the entire sequence of the 5'-noncoding region.

SDH from rat liver catalyzes dehydration of L-serine as well as L-threonine, to ammonia and the corresponding keto acids [1]. Similar enzymes were found in *E. coli* and yeast; namely biodegradative (catabolic) threonine dehydratase, biosynthetic L-threonine dehydratase and D-serine dehydratase. Recently, Datta et al. [13] cloned the gene for the biodegradative threonine dehydratase of *E. coli* and reported that the deduced amino acid sequence of this gene showed significant homology with those of biosynthetic threonine dehydratase of yeast [14] and D-serine dehydratase of *E. coli* [15], despite their diverse origins and metabolic significances.

Alignment of the deduced amino acid sequence

of the rat liver SDH with those of the biosynthetic and biodegradative threonine dehydratases from yeast and *E. coli*, respectively, and *E. coli* D-serine dehydratase indicated lower homology of SDH with D-serine dehydratase than with the threonine dehydratases. As shown in fig.3, there are several homologous domains in the primary structures of SDH, and biosynthetic and biodegradative threonine dehydratases. Despite of differences in metabolic significance, the overall homology between the yeast biosynthetic threonine dehydratase and *E. coli* biodegradative threonine dehydratase is higher than that between the rat SDH and *E. coli* biodegradative threonine dehydratase. Highly conserved sequences were located at amino acids 38–44, and 167–172 in SDH. The former is the heptapeptide ³⁸Gly-Ser-Phe-Lys-Ile-Arg-Gly⁴⁴, which is exactly the same as that of *E. coli* biodegradative threonine dehydratase. The heptapeptide from yeast biosynthetic threonine dehydratase is also conserved except for a difference of one amino acid residue, if the replacement of isoleucine by leucine is regarded as the conserved sequence. These domains in the biosynthetic and biodegradative threonine dehydratases are identified as active sites for binding of pyridoxal

phosphate and contain a conserved lysine at the same positions. Since SDH from rat liver also contains Lys-41, this region appears to be the binding site for pyridoxal phosphate. However, the sequence is not homologous with those of other pyridoxal phosphate-dependent enzymes summarized by Bossa et al. [16], such as serine transhydroxymethylase from rabbit liver, amino acid decarboxylase from *E. coli*, tryptophanase and tryptophan synthase in bacteria, and amino acid transaminases in mammals and bacteria, although significant homology is observed in a group of enzymes catalyzing a similar reaction. The other highly conserved sequence is 14-peptides located from Ile-163 in SDH. The sequences in the three enzymes contain 4 glycine residues at the center with mainly hydrophobic amino acids before and after this sequence. The functional significance of this domain is unclear, but this domain, in conjunction with the pyridoxal phosphate binding site, may be the active site for the dehydration reaction as proposed previously [13]. It is noteworthy that the heptapeptide and 14-peptide sequences are also conserved in D-serine dehydratase of yeast [13,15].

Other homologous domains are between His-59

SDH	25	T	S	V	F	L	K	M	D	S	S	Q	P	S	G	S	F	K	I	R	G	I	G	H	47													
YBTD	93	T	N	V	I	L	K	R	E	D	L	L	P	V	P	S	F	K	L	R	G	A	Y	N	116													
ETD	42	G	E	I	F	L	K	F	E	N	M	Q	R	T	G	S	F	K	I	R	G	A	F	N	65													
SDH	59	H	F	V	I	C	S	S	A	G	N	-	A	G	M	A	T	A	Y	A	A	R	R	L	G	L	P	A	T	T	V	V	P	S	T	T	P	92
YBTD	128	G	V	I	A	C	S	S	A	G	N	H	A	Q	G	V	-	A	F	A	A	K	H	L	K	I	P	A	T	I	V	M	P	V	C	T	P	162
ETD	77	G	V	V	A	C	S	S	A	G	N	H	A	Q	G	V	-	S	L	S	C	A	M	L	G	I	D	G	K	V	V	M	P	K	G	A	P	110
SDH	120	A	K	A	L	E	K	N	N	Q	G	W	V	Y	I	S	P	F	D	D	P	L	I	W	E	G	H	T	F	L	V	K	E	I	152			
YBTD	190	A	K	L	A	E	E	-	-	R	G	L	T	N	I	P	P	F	D	H	P	V	V	I	A	G	Q	G	T	V	A	M	E	I	220			
ETD	139	S	E	I	V	E	M	-	-	E	G	R	I	F	I	P	P	Y	D	D	P	K	V	I	A	G	Q	G	T	I	G	L	E	I	169			
SDH	163	I	V	L	S	V	G	G	G	G	L	L	C	G	V	176																						
YBTD	233	V	F	V	P	V	G	G	G	G	L	I	A	G	I	246																						
ETD	179	V	I	V	P	I	G	G	G	G	L	I	A	G	I	192																						

Fig.3. Homology of the amino acid sequences of SDH with those of yeast biosynthetic threonine dehydratase (YBTD) [14] and *E. coli* biodegradative threonine dehydratase (ETD) [15]. The sequences are aligned to obtain maximal homology. Homologous residues between polypeptides are boxed.

and Phe-92, and between Ala-120 and Ile-152 in SDH. The former domain shows 56% and 35% homology, and the latter 40% and 31%, with the corresponding domains of yeast biosynthetic threonine dehydratase and *E. coli* biodegradative threonine dehydratase, respectively. The pentapeptide ⁶⁴Ser-Ala-Glu-Asn-Ala⁶⁸ of SDH is well conserved, but is not found in D-serine dehydratase [13,15]. The rest of the sequences in these domains were also not homologous to those of D-serine dehydratase. Very little homology is found in the C-terminal halves of these enzymes: the homologous domains in SDH are located within the N-terminal half. The function of the remaining C-terminal region is unknown.

REFERENCES

- [1] Nakagawa, H. and Kimura, H. (1969) *J. Biochem.* 66, 669–683.
- [2] Inoue, H., Kasper, C.B. and Pitot, H.C. (1971) *J. Biol. Chem.* 246, 2626–2632.
- [3] Snell, K. (1986) *Trends Biochem. Sci.* 11, 241–243.
- [4] Noda, C., Tomomura, M., Nakamura, T. and Ichihara, A. (1984) *J. Biochem.* 95, 37–45.
- [5] Nakamura, T., Niimi, S., Nawa, K., Noda, C., Ichihara, A., Takagi, Y., Anai, M. and Sakaki, Y. (1987) *J. Biol. Chem.* 262, 727–733.
- [6] Noda, C., Tomomura, M., Nakamura, T. and Ichihara, A. (1985) *Biochem. Biophys. Res. Commun.* 132, 232–239.
- [7] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: *Molecular Cloning; A Laboratory Manual*, pp.329–333, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- [8] Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* 33, 103–119.
- [9] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- [10] Proudfoot, N.J. and Brownlee, G.G. (1978) *Nature* 263, 211–214.
- [11] Konkel, D.A., Tilghman, S.M. and Leder, P. (1978) *Cell* 15, 1125–1132.
- [12] Kozak, M. (1984) *Nucleic Acids Res.* 12, 8857–8872.
- [13] Datta, P., Goss, T.J., Omnaas, J.R. and Patil, R.V. (1987) *Proc. Natl. Acad. Sci. USA* 84, 393–397.
- [14] Kielland-Brandt, M.C., Holmberg, S., Peterson, J.G.L. and Nilsson-Tillgren, T. (1984) *Carlsberg Res. Commun.* 49, 567–575.
- [15] Schlitz, E. and Schmitt, W. (1981) *FEBS Lett.* 134, 57–62.
- [16] Bossa, F., Barra, D., Martini, F., Schirch, L.V. and Fasella, P. (1976) *Eur. J. Biochem.* 70, 397–401.