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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). T_4 polynucleotide kinase and T_4 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-^{32}P]CTP and [γ-^{32}P]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

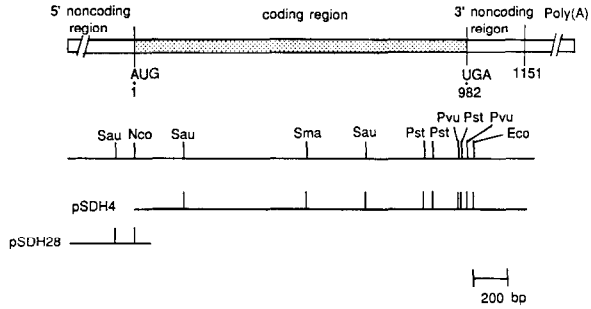

Fig.1. Restriction endonuclease cleavage sites of the SDH cDNA inserts and organization of SDH mRNA. The numbers below the structure of the mRNA indicate the length in nucleotides from AUG to termination codon UAG and to the poly(A) addition site. Sau, *Sau*3A; Nco, *Nco*I; Sma, *Sma*I; Pst, *Pst*I; Pvu, *Pvu*II; Eco, *Eco*RV. 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 ^{49}ATG^{51} or ^{91}ATG^{93}. 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 ^{1}ATG^{3} is the first initiation codon except $^{-5}$ATG^{-3}. But, it is unlikely that $^{-5}$ATG^{-3} is the

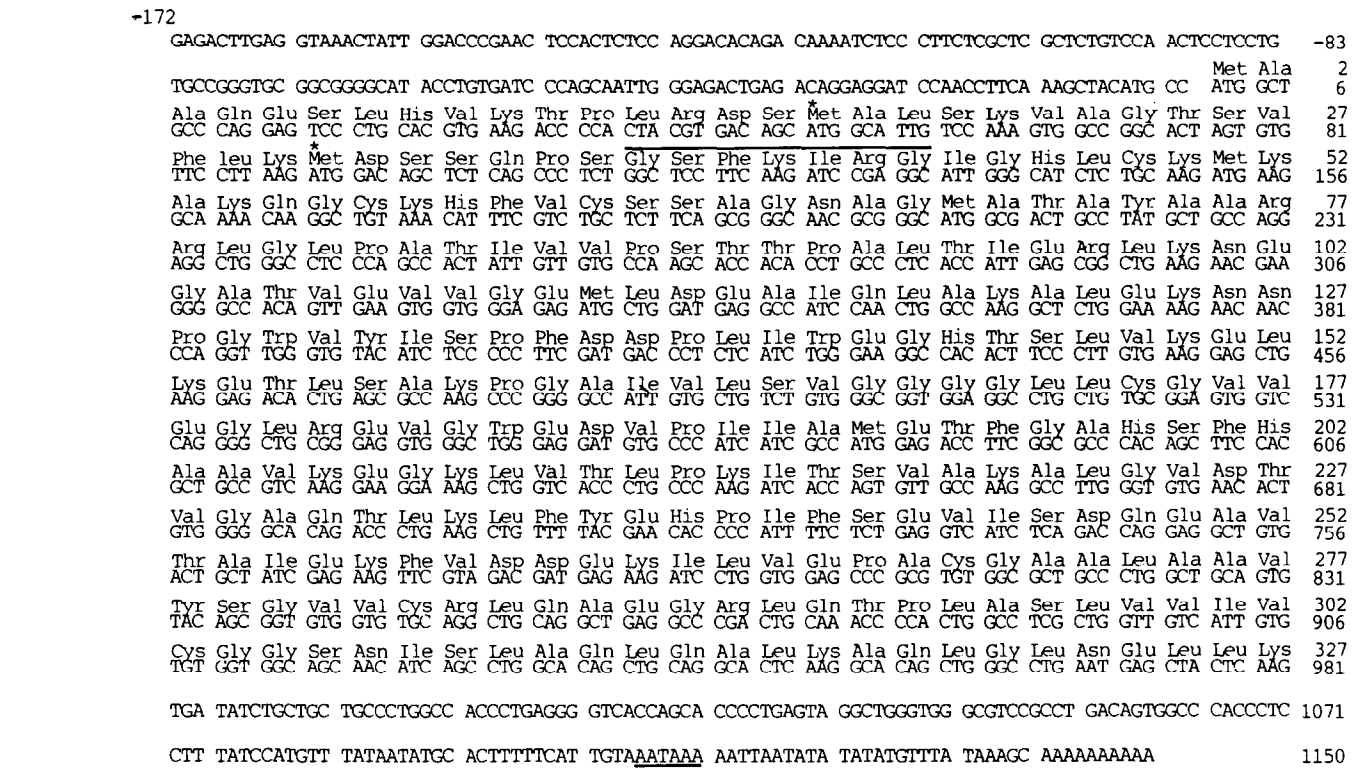

Fig.2. Nucleotide sequence of the SDH cDNA and the deduced amino acid sequence of SDH. The upper numbers on the right represent amino acid residues of the polypeptide from the N-terminus. Nucleotides in the SDH sequence are numbered from the translational initiation site (lower numbers). The predicted 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 $^{-5}CC^{A}_{G}CC^{-1}$ just upstream from the initiation codon is reported to be conserved [12]. The sequence in SDH cDNA, $^{-5}$ATGCC^{-1}, partly matches this conserved sequence. If ^{1}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 ^{38}Gly-Ser-Phe-Lys-Ile-Arg-Gly^{44}, 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

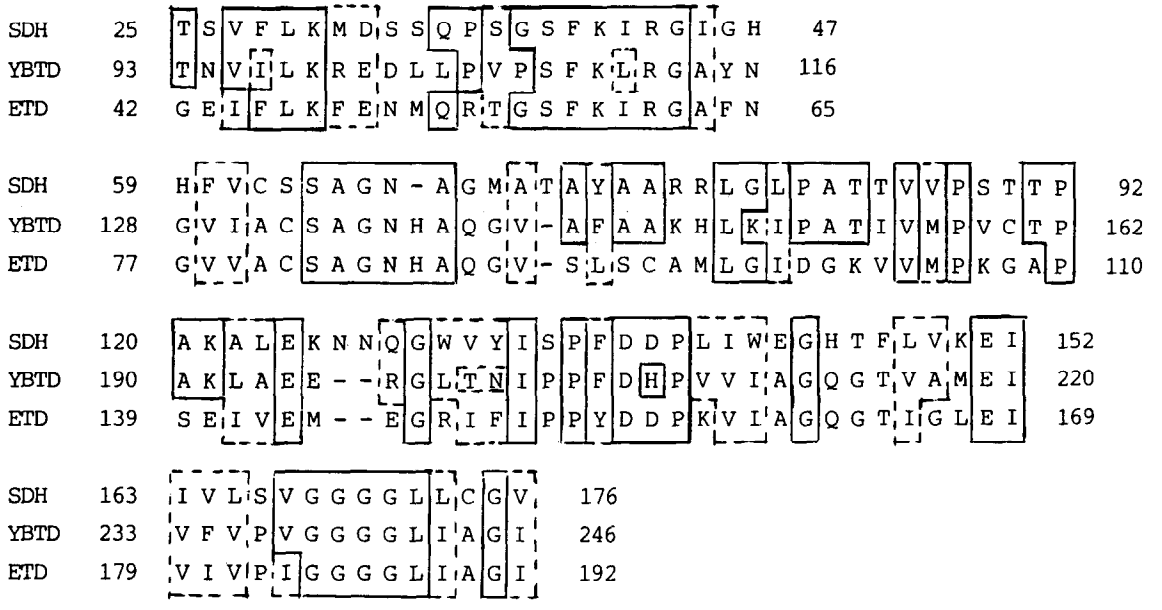

Fig.3. Homology of the amino acid sequences of SDH with those of yeast biosynthetic threonine dehydratase (YBTD) [14] and *E. coli* biodegradative threonine dehydratase (ETD) [15]. The sequences are aligned to obtain maximal homology. Homologous residues between polypeptides are boxed.

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 ^{64}Ser-Ala-Glu-Asn-Ala^{68} 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.

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