Molecular structure of the human alcohol dehydrogenase 3 gene*†

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ABSTRACT

The structure and nucleotide sequence of an ADH3¹ allele, which encodes the ADHγ₁ subunit, have been determined. The intron positions of the ADH3 gene are identical to those of the other class I and class II ADH genes. The level of nucleotide variation at the ADH3 locus is somewhat higher than those at the ADH1 and ADH2 loci.

1. INTRODUCTION

Human class I alcohol dehydrogenase (ADH) consists of the subunits α, β, and γ, which are encoded by three separate loci ADH1, ADH2, and ADH3, respectively (e.g., see Smith, 1986). These three loci are located tandemly on chromosome 4 (Yasunami et al., 1990). Molecular analyses have been conducted for the ADH1 (Matsuо and Yokoyama, 1989) and ADH2 (Duester et al., 1986; Matsuо et al., 1989) genes, showing that 9 exons are stretched over 15 kilobases (kb) in length.

At the ADH3 locus, only the complementary DNA sequences of two common alleles have been characterized (Ikuta et al., 1986; Hoog et al., 1986). Here, we report the intron/exon structure and the DNA sequence of the coding region of a human ADH3¹ allele, which encodes the ADH γ₁ subunit.

2. MATERIALS AND METHODS

Two sets of genomic libraries were constructed by using human genomic DNA from one of us (SY) which had been partially digested either with Mbo I or Eco RI

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† DDBJ accession number: The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers D11059-D11067.
and ligating with \( \lambda EMBL3 \) or \( \lambda EMBL4 \) DNA, respectively (Matsuo and Yokoyama, 1989; Matsuo et al., 1989). Out of 16 clones which have been classified as ADH3 gene (Matsuo and Yokoyama, 1990) one clone SY\( \lambda ADH15 \), of size about 14.5 kb, was analyzed in detail. Subcloning was initially conducted by ligating \( EcoRI \)-, \( HindIII \)-, or \( BamHI \)-digested cloned DNA into the plasmid Bluescript vector from Stratagene. Further subclones were made either by using other restriction enzyme digests or exonuclease III/mung bean nuclease deletions as described by Stratagene. They were sequenced by using the dideoxy-chain-termination method (Sanger et al., 1977; Hattori et al., 1985).

SY\( \lambda ADH15 \) contains part of intron 1 through the 3\(^\prime\)-flanking region and we could not identify clones which contain the exon 1 of the ADH3 gene. Thus, to determine the DNA sequence of exon 1 and its surrounding regions, amplification of the genomic DNA was performed using the polymerase chain reaction (PCR). The amplification was carried out using the GeneAmp Kit and thermal cycler of Perkin Elmer-Cetus. The reaction included 100 ng of the genomic DNA from SY as template and two oligonucleotide primers (5\(^\prime\)-CCAAATGCACCTCAAGCA-GAGAAG-3\(^\prime\) and 5\(^\prime\)-CCTGAAGTCTCAAAGCTGCTGTC-3\(^\prime\)). The first primer is based on the sequence in the 5\(^\prime\)-flanking region about 50 bp upstream of the initiation codon of cDNA ADH \( \gamma_1 \) (Ikuta et al., 1986). The second primer is also ADH3 gene-specific and based on the sequence information in the intron 1 region, which was derived from the clone SY\( \lambda ADH15 \). The reaction was carried out for 29 cycles in which DNA was denatured at 94\( ^\circ \)C for 45 sec, annealed at 55\( ^\circ \)C for 45 sec, and extended at 72\( ^\circ \)C for 3 min.

The following modifications (Barry Hall, pers. comm.) allowed sequencing of genomic DNA from PCR-amplified DNA directly. The amplified DNA was extracted with chloroform:isoamyl alcohol (24:1) and then purified by using GENECLEAN II Kit (BIO 101, Inc.) according to the manufacturer’s instruction. One-half of the amplified product was denatured in the presence of annealing buffer (Sequenase kit, U.S. Biochemicals) and the first primer by boiling 5 min and then placing in dry ice for 5 min. The sequencing reaction was then carried out following the Sequenase kit instructions, except that the times for room temperature incubation and 37\( ^\circ \)C termination steps were decreased to 30–45 sec.

3. RESULTS AND DISCUSSION

The DNA sequence of all nine exons of the ADH3\(^1\) allele is shown in Fig. 1. Like the ADH1 and ADH2 genes, the ADH3 gene is also divided by eight introns. The positions of the introns are identical to those of the ADH1 and ADH2 genes. The approximate sizes of the introns 1–8 of the ADH3 gene are 4.5, 0.6, 1.9, 0.1, 1.9, 2.2, 0.7, and 2.3 kb, respectively. With the exception of the intron 1, their lengths are very similar to those of the ADH1 (Yasunami et al., 1990) and ADH2 (Duester et al., 1986; Matsuo and Yokoyama, 1989) genes. The intron 1 of the
Fig. 1. DNA sequence of a human ADH3 gene. The DNA sequence of all nine exons is shown with the predicted 374 amino acids. The size of the eight introns are indicated.
ADH3 gene is about 1 kb longer than those of ADH1 (3.2 kb) and ADH2 (2.8 kb) genes.

It should be noted that the class II (ADH4) gene also has 8 introns at identical sites to the class I ADH genes (Von Bahr-Lindstrom et al., 1991) and the lengths of introns 1–8 are 1.8, 0.9, 2.6, 2.7, 5.5, 3.5, 0.5, and 2.6 kb, respectively (Von Bahr-Lindstrom et al., 1991). Thus, the intron 1 of the ADH4 gene is about a half of those of the class I genes, whereas the introns 4 and 5 of the ADH4 gene are much longer than those of the class I ADH genes.

At the ADH3 locus, two common alleles have been identified: ADH3\(^1\) and ADH3\(^2\) which encode the ADH \(\gamma_1\) and \(\gamma_2\) subunits, respectively (Smith, 1986). Amino acid sequences of the \(\gamma_1\) and \(\gamma_2\) differ only at two positions. That is, ADH \(\gamma_1\) consists of arginine and isoleucine at the 271 and 349 residues, respectively, whereas the respective amino acids for ADH \(\gamma_2\) are glutamine and valine (Hoog et al., 1986). Clearly, SY\(\lambda\)ADH15 represents the ADH3\(^1\) allele (Fig. 1; Table 1). When the three available ADH3\(^1\) alleles are compared, we find three silent substitutions among them (Table 1). The level of nucleotide variation at the ADH3 locus is somewhat higher than those at the ADH1 (Matsuo and Yokoyama, 1989) and ADH2 (Matsuo et al., 1989) loci, where no silent substitution has been detected. Table 1 strongly suggests that nucleotides T, T and G at the third codon positions of the residues 100, 103, and 150, respectively, are the latest changes.

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REFERENCES
