Molecular structure of the human alcohol dehydrogenase 1 gene

Yoshinori Matsuo and Shozo Yokoyama

Department of Ecology, Ethology, and Evolution, University of Illinois at Urbana-Champaign, Shelford Vivarium, 606 East Healey Street, Champaign, IL 61820, USA

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The structure and nucleotide sequence of an allele at the ADH1 locus have been determined. The nucleotide sequence of this allele is identical to that of a cDNA clone ([1986] Biochemistry 25, 2465-2470) and the intron positions of the ADH1 gene are identical to that of the ADH2 gene ([1986] J. Biol. Chem. 261, 2027-2033).

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 [1]. Molecular characterization of the ADH2 gene shows that 9 exons are stretched over 15 kilobases (kb) in length [2,3]. However, the molecular structures of the ADH1 and ADH3 loci have not been determined.

The complementary DNA sequences of the ADH1 alleles isolated from two different individuals were identical [4,5]. To study the genomic structure and level of DNA polymorphism at the ADH1 locus, we determined the DNA sequence of all nine exons and the intron/exon structure of a human ADH1.

2. MATERIALS AND METHODS

Two sets of genomic libraries were constructed by using genomic DNA partially digested either with MboI or EcoRI and ligating with λEMBL3 or λEMBL4 DNA, respectively. About 120 µg of the genomic DNA from one of us (S.Y.), obtained from peripheral blood leukocytes [6], was partially digested and fractionated on an agarose gel. The DNA in the size range of 9-23 kb was electrophoresed from the gel and ligated with λEMBL vector DNA which had been double digested with BamHI and EcoRI. The ligated DNA was packaged in vitro into phage particles by using Gigapack packaging extract (purchased from Stratagene) and plated on the nonpermissive E. coli host NM539.

Plaque hybridization was carried out using the method of Benton and Davis [7] for 24–36 h at 68°C in 4 x SETDS with nick-translated [8] cDNA probe of ADHβ [9] and 50 µg/ml of heat denatured herring sperm DNA (4 x SETDS: 0.6 M NaCl (pH 7.5), 8 mM EDTA, 10 x Denhardt, 0.1% SDS). From the two human genomic libraries, a total of one million recombinant plaques were screened.

Oligonucleotides which are specific for the subunits α, β, and γ between amino acids 312 and 320 were synthesized and used for locus specific hybridization. Hybridization was carried out with the γ-32P-end-labelled oligomers and heat denatured herring sperm DNA at 37°C for 12 h in 4 x SETDS [3].

Subcloning was conducted using restriction enzymes BamHI, EcoRI, HindIII, KpnI, NsiI, PstI, Sau3A, SpeI, StuI and XbaI. Digested DNA fragments were ligated into the plasmid Bluescript vector from Stratagene. They were sequenced by using the dideoxy-chain-termination method [10–12].

3. RESULTS AND DISCUSSION

For 63 positive clones, restriction mapping and locus specific oligonucleotide hybridization were conducted. The numbers of clones which belong to
the ADH1, ADH2, and ADH3 loci were 17, 14, and 13, respectively. The remaining 19 positive clones have not been characterized yet.

To determine the complete nucleotide sequence of all nine exons of the ADH1 gene, two overlapping clones SYAADHE17 and SYAADH16 were used (fig.1). Hybridization experiments showed that 10.7 kb SYAADHE17 had exons 1–6, whereas 16 kb SYAADH16 had exons 6–9. Evidence that the two clones were derived from the same allele comes from a restriction site polymorphism within intron 6. Four out of ten overlapping clones studied have a TaqI site about 350 bp downstream from exon 6, but the remaining six clones, including SYAADHE17 and SYAADH16, did not have that site.

The DNA sequence of all nine exons of the ADH1 allele is shown in fig.2. When this sequence was compared to the published cDNA sequences of two different ADH1 alleles [4,5], no nucleotide difference in the coding region was found. Similarly, a very low level of genetic variability has been found between the two different electrophoretic alleles at the ADH2 locus [3,13].

Fig.2 also shows that the ADH1 gene is divided by eight introns (see also fig.1). The positions of the introns are identical to those of the ADH2 gene. Nine exons of the ADH1 gene are stretched over about 15 kb in length and the approximate sizes of the intron 1–8 of the ADH1 gene are 3.2, 0.6, 1.8, 0.1, 1.9, 2.2, 0.6 and 2.8 kb, respectively. The corresponding introns of the ADH2 gene are 2.8, 0.6, 1.7, 0.1, 2.0, 2.2, 0.6 and 2.8 kb, respectively [2,3]. These sizes are very similar and the intron/exon structure of the ADH1 and ADH2 loci are well conserved since their divergence.

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Fig.2. DNA sequence of a human ADH1 gene. The DNA sequence of all nine exons is shown with the predicted 374 amino acids. The sizes of the eight introns are indicated.

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REFERENCES