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Genotyping of human class I alcohol dehydrogenase

Analysis of enzymatically amplified DNA with allele-specific oligonucleotides

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Large inter-individual differences are noted in the susceptibility to alcohol-related problems. Part of this variation may be due to the different isoenzyme patterns of the alcohol-metabolizing enzymes and, consequently, different pharmacokinetics of alcohol degradation. We have used the polymerase chain reaction and oligonucleotide hybridization to amplify and analyze class I alcohol dehydrogenase isoenzyme-specific genomic DNA. The method unambiguously distinguishes between different allelic variants and thus provides a new means of elucidating the alcohol dehydrogenase isoenzyme pattern of humans.

Alcohol dehydrogenase; Genotyping; Polymerase chain reaction; Allele-specific oligonucleotide

1. INTRODUCTION

Our laboratory has a long-standing interest in the enzymes involved in alcohol metabolism. In particular, we are interested in elucidating the potential role of these enzymes with regard to the various pathological consequences of alcohol consumption, which are intimately correlated to the tissue concentration of ethanol and its metabolic products. The rate-limiting step in the metabolism of ethanol is its oxidation to acetaldehyde, primarily catalyzed by isoenzymes of class I alcohol dehydrogenase (ADH, EC 1.1.1.1). The enzyme consists of a mixture of isoenzymes: three subunits, α, β and γ, encoded by the genes ADH_1, ADH_2 and ADH_3 combine randomly to form enzymatically active homo- and heterodimers [1-3]. Polymorphism is observed at ADH_2 and ADH_3, giving rise to three β-subunits (β_{1-3}) [2,4] and to two γ-subunits (γ_1, γ_2) [2,3]. Class I ADH isoenzymes show strict sequence homology [5] but substantial dissimilarities in their overall catalytic rate are observed [6]. Enzymatic differences are mainly expressed by the allelic variants encoded at ADH_2 and ADH_3 and can be explained by defined residue exchanges [7].

To date, the isoenzyme pattern of an individual could only reliably be determined in organ biopsy and autopsy material by electrophoretic and kinetic identification of the phenotype [3,8,9], which prevented epidemiological studies. Recent advances in molecular biology have provided a means of determining the genotype of an individual.

Here, we show that the class I ADH genotype at the ADH_2 and ADH_3 loci can be determined using allele-specific oligonucleotides and DNA sequences amplified in vitro by the polymerase chain reaction (PCR) technique [10,11].

This work is dedicated to the memory of Susanne Schindler

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Abbreviations: ADH, alcohol dehydrogenase; PCR, polymerase chain reaction

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2. EXPERIMENTAL

Genomic DNA was extracted from the livers of kidney donors or from autopsy. For each liver the class I ADH phenotype was determined. The presence of isoenzymes containing β_1- and β_2-subunits was investigated by examining the pH dependence of ethanol oxidation [8] and of isoenzymes containing γ_1- and/or γ_2-subunits through electrophoretic analysis of homogenate supernatants [3]. Cloned DNA of known sequence was used as a control. The genomic clone pADH36 contains exon 3 and surrounding regions of the β_1 ADH gene [12]; the cDNA clone λADH6 was isolated in our laboratory and codes for 1 kb of the β_2 mRNA.

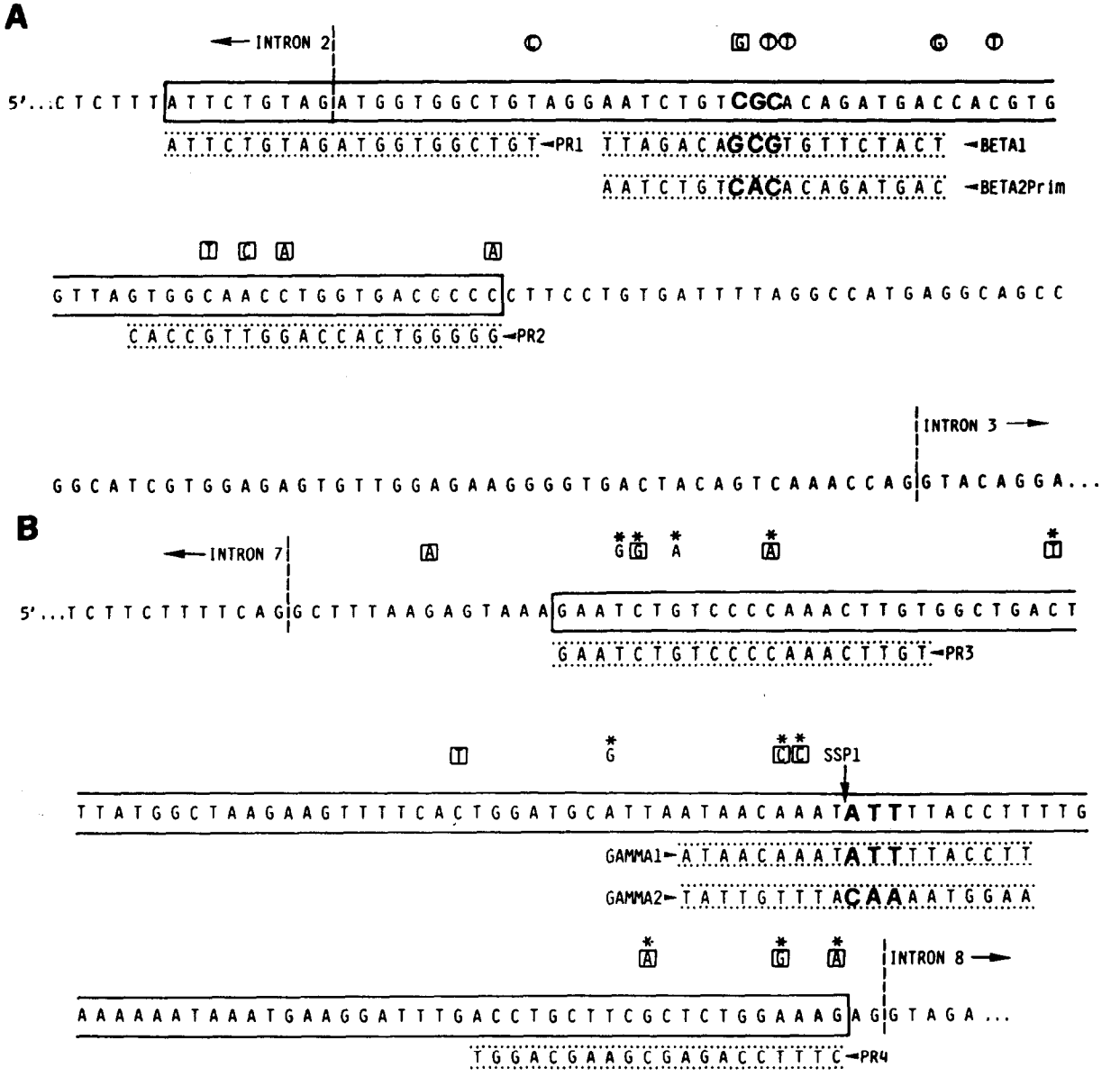

Fig.1. Rationale for genotyping human class I ADH. (A) Exon 3 and parts of introns 2 and 3 of the β_1 gene. (B) Exon 8 and parts of the adjoining introns of the γ_1 gene. Codons for position 47 of the β_1-subunit and position 349 of the γ_1-subunit are in bold type. Differences in coding sequences of the α [19], β [16] and γ [18] subunits are shown by □, ★ and ○, respectively. Sequences and positions of the PCR primers PR1–PR4 and of the allele-specific oligonucleotides BETA1, BETA2Prim, GAMMA1 and GAMMA2 are indicated. Again relevant nucleotides are in bold type. The 72 bp and 123 bp DNA fragments are boxed.

Allele-specific oligonucleotides and primers for the PCR were synthesized by the solid-phase triester method and purified on polyacrylamide gels [13,14]. Their sequences are shown in fig. 1. The oligomers were end-labeled to a specific activity of ~ 10^8 dpm/μg using [γ-^{32}P]ATP (Amersham, England) and T_4-polynucleotide kinase (Boehringer, Mannheim, FRG) [13].

The PCR was performed following Saiki et al. [11]. With genomic DNA annealing and extension steps were performed at 40°C. Starting material was 1 μg liver DNA and 25 PCR cycles were carried out. Amplified DNA preparations (aliquots containing 0.5 μg of the original DNA) were separated on 8% polyacrylamide gels [13] and electrophoretically transferred to Zeta-Probe nylon membranes (Bio-Rad, Richmond, CA) following the instructions of the manufacturer. Alternatively, aliquots containing 0.5 μg of the original DNA were applied to Zeta-Probe membranes under vacuum, using a Minifold II slot-blotter (Schleicher and Schuell, Dassel, FRG). Filters were prehybridized in 5 × SSPE (1 × SSPE: 10 mM NaH_2PO_4, 0.18 M NaCl, 1 mM EDTA, pH 7.4), 5 × Denhardt's (1 × Denhardt's: 0.02% each of bovine serum albumin, polyvinylpyrrolidone and Ficoll) and 0.5% SDS for 2–5 h. Hybridization was performed in the same solution containing 4.5 × 10^6 dpm/ml of end-labeled oligonucleotide (10–50 ng), for 5 h or overnight. Hybridization temperatures for the PR3, BETA 1, BETA2Prim, GAMMA1 and GAMMA2 oligomers were 58, 56, 54, 44 and 46°C, respectively. Filters were rinsed in 5 × SSPE at room temperature (2 × 15 min), followed by a high-stringency wash in 2 × SSPE, 0.1% SDS (2 × 15 min) at 60, 56, 54, 46 and 48°C, respectively, and autoradiographed at −70°C.

3. RESULTS AND DISCUSSION

Many recent publications have demonstrated the reliability and usefulness of the PCR technique for analysis of allelic variants by sequence-specific oligonucleotide probe hybridization (e.g. see [15,16]). We have applied this method to the β_1, β_2, γ_1 and γ_2 alleles of human class I ADH. Fig.1 shows the relevant sequences of the β and γ ADH genes. Exon 3 of the β_1 gene contains the triplet CGC coding for Arg-47 [17] (fig.1A). This residue is exchanged for a His in the β_2-subunit [18]. DNA preparations were amplified using the PCR primers PR1 and PR2, resulting in amplified fragments of 72 bp (fig.1A). Exon 8 of the γ gene contains the codon for position 349, which is Ile (ATT) in the γ_1-subunit and Val (GTT) in the γ_2-subunit [19] (fig.1B). Amplification of the DNA defined by primers PR3 and PR4 generated a fragment of 123 bp (fig.1B).

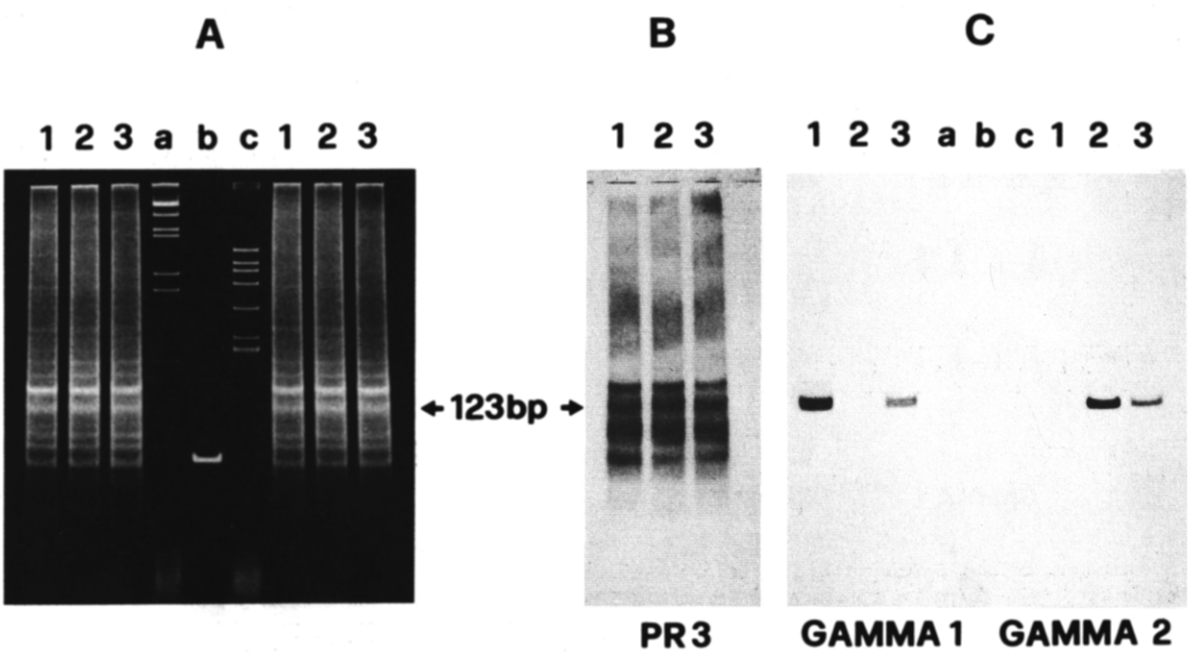

Fig.2. Analysis of DNA amplified in vitro to enrich a defined DNA fragment from the γ ADH gene. (A) Polyacrylamide gel, stained with ethidium bromide; (B) electroblot probed with the PCR primer PR3; (C) electroblots probed with the allele-specific oligonucleotides GAMMA1 and GAMMA2. Lanes 1–3, amplified DNA preparations from $\gamma_1\gamma_1$, $\gamma_2\gamma_2$ and $\gamma_1\gamma_2$ phenotypes, respectively. Lanes a–c, size markers, pB40/*Hind*III [21], 72 bp DNA fragment amplified from pADH36, and pBR322/*Alu*I, respectively. The position of the 123 bp DNA fragment amplified from the γADH gene is indicated by arrows.

The formation of defined DNA fragments was confirmed by polyacrylamide gel electrophoresis of amplified DNA preparations, followed by hybridization analysis using the PCR primers and the allele-specific oligonucleotides. As shown in fig.2 for the γ-system numerous sequences were amplified by the PCR (A) and hybridized with the PCR primers (B). This nonspecificity is due to the low temperature required for polymerase activity which allows nonspecific priming at multiple sites along the genomic DNA. The allele-specific oligonucleotides GAMMA1 and GAMMA2 specifically and selectively hybridized with the expected 123 bp fragment (fig.2C). Analogously, the β-system yielded multiple fragments of which only the expected 72 bp fragment hybridized with the BETA1 and BETA2Prim probes. Since no DNA from homozygous $\beta_2\beta_2$ individuals was available, the specificity of the two probes was assessed by hydridization with amplified DNA preparations from the β_1- and β_2-specific clones pADH36 and λADH6, respectively (fig.3A).

The reliability of the method was verified by applying it to liver specimens of known ADH isoenzyme composition. DNA was extracted from 20 human livers, amplified in vitro and analyzed by slot-blot hybridization. The results are shown in fig.3. The class I ADH genotype of each liver could clearly be determined and in each case agreed with the previously established phenotype. DNA of two livers was amplified using all four primers simultaneously. In both cases the β- and γ ADH-genotype could be determined unambiguously. Work is now in progress to apply the procedure to DNA extracted from blood samples. The PCR and subsequent hybridization of the DNA fragments generated with allele-specific oligonucleotide probes will provide a means for genotyping a large number of individuals with respect to their class I ADH isoenzyme pattern.

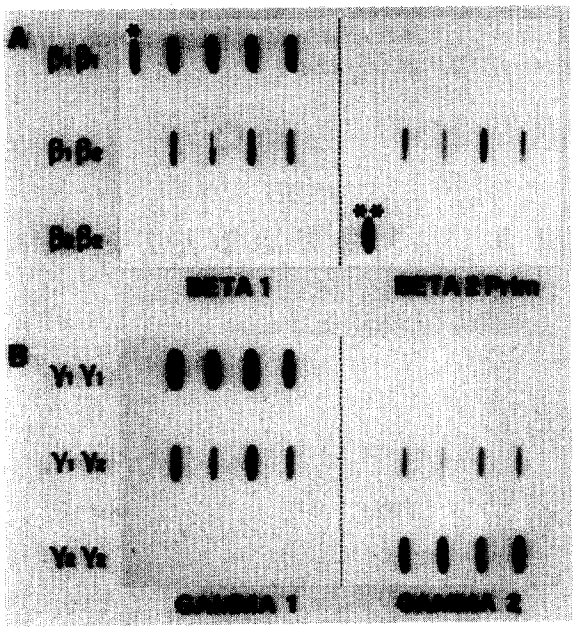

Fig.3. Genotyping of human class I ADH. (A) DNAs tested were from $\beta_1\beta_1$ and $\beta_1\beta_2$ livers and from the β_1- and β_2-specific clones pADH36 (★) and λ ADH6 (★★). Amplification was directed by the PCR primers PR1 and PR2. Genotyping was carried out with the ADH_2 locus-specific oligonucleotides BETA1 (left) and BETA2Prim (right). (B) DNAs tested were from $\gamma_1\gamma_1$, $\gamma_1\gamma_2$ and $\nu_2\nu_2$ livers. Amplification was directed by the PCR primers PR3 and PR4. Genotyping was carried out with the ADH_3 locus-specific oligonucleotides GAMMA1 (left) and GAMMA2 (right).

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