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UPSTREAM STRUCTURE OF HUMAN *ADH7* GENE AND THE ORGAN DISTRIBUTION OF ITS EXPRESSION

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SUMMARY The *ADH 7* gene encoding human class IV (σ) alcohol dehydrogenase (ADH) was cloned from a Caucasian genomic DNA library and its upstream structure was determined. Moreover, the organ distribution of its expression was examined. Northern hybridization analysis with a specific probe for σ-ADH showed that expression of the gene is organ specific rather than ubiquitous, and occurs in the stomach but not in the liver. The lack of CG rich sequence and presence of TATA and CCAAT boxes in the upstream region of *ADH7* may reflect the organ specific expression. The findings that this region lacks hepatocyte nuclear factor and has only one CCAAT enhancer binding protein consensus site may account for the fact that this gene is not expressed in the liver. The upstream region had sequences which are compatible with a glucocorticoid response element, a metal binding factor-1, and an active gene regulatory protein-1, suggesting that sex hormones, zinc, and retinoic acid may be involved in the regulation of the expression of this gene.

Human alcohol dehydrogenase (ADH, EC 1.1.1.1.) comprises a family of enzymes that participate in oxido-reduction between various alcohols and the corresponding aldehydes (1). Until recently, five protein subunits (α, β, γ, π, χ) which combine into three classes of isoenzymes [Class I (α, β, γ) , Class II (π) and Class III (χ) ADHs] have been identified (2-3). The corresponding genes, namely *ADH1* (4) , *ADH2* (5-7), *ADH3* (8), *ADH4* (9), and *ADH5* (10), have also been fully characterized. Moreover, an additional ADH gene, *ADH 6* (11), has been cloned and classified as Class V ADH, but so far its protein subunit has not been found *in vivo*.

Recent studies have identified another isoenzyme of ADH in the human stomach (12-14) designated as Class IV or σ ADH. Observations on electrophoresis indicate that this isoenzyme is expressed in the upper digestive tract rather than in the liver (13, 14). This organ distribution is different from that of Class I ADH isoenzymes which play a major role in

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ethanol metabolism in the liver, and that of Class III ADH that is ubiquitously expressed in most organs. The Class IV enzyme is believed to contribute to the decreased bioavailability of imbibed alcohol because of gastric first-pass metabolism, which may represent an important barrier against ethanol toxicity (15). In addition, due to its substrate specificity (13, 16,17), this ADH may be involved in the metabolism of substances other than ethanol. More recently, its cDNA was cloned and its entire structure determined by several groups (17-19), including our own (20). Northern hybridization analysis with a specific probe for σ ADH mRNA showed no expression of its mRNA in the liver (20).

The aims of this study were to clone the gene encoding σADH, namely *ADH7*, to extend studies on the organ distribution of the mRNA expression, and to search in the upstream region of the gene for response elements that could be involved in the regulation of its expression.

MATERIALS AND METHODS

Molecular cloning of *ADH 7* gene and sequencing.

The molecular cloning of *ADH7*, the purification of DNA of the bacteriophage carrying *ADH7* and its sequencing were described in detail elsewhere (21). The sense and antisense sequencing primers which identified each exon of *ADH7* were designed from the cDNA sequence of human Class IV ADH (20) and the reported exon/intron structure of *ADH2* (5). After sequencing, additional primers (UP1S, UP2S, and UP1AS, Figure 1) were designed based on the nucleotide sequences obtained, in order to determine the structure of the upstream region of *ADH7*. Transcriptional factors in the upstream region were searched by Mac Vector (version 4.0.1; Eastman Kodak Inc., Rochester NY) with a Macintosh IICi computer.

Organ distribution of the *ADH 7* gene expression.

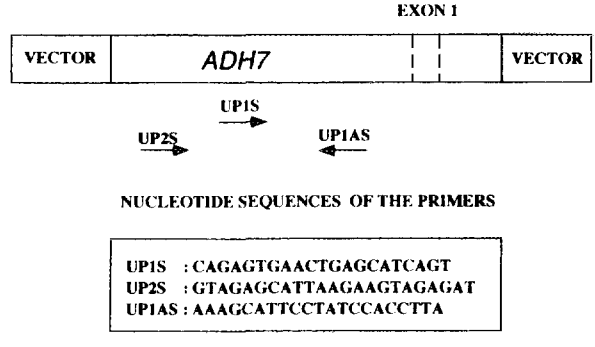

Fig 1. Strategy for the characterization of ADH7 upstream. The nucleotide sequences of primers used for sequencing are shown. Arrows indicate the direction of the sequencing with these primers.

To examine the organ distribution of the *ADH 7* gene expression, northern hybridization analysis was carried out with the [α-^{32}P] dCTP (DuPont NEN, Boston MA) labeled specific probe for human σ ADH mRNA previously constructed (20). Pre-blotted mRNA from a variety of human tissues (Multiple Tissue Northern Blots) were purchased from Clontech Lab. Inc. (Palo Alto, CA). In addition, mRNA was prepared from total RNA of human gastric mucosa (20) by affinity chromatography in an oligo dT cellulose column (22) (Sigma Chemical Co., St. Louis, MO). Two μg of the samples were subjected to RNA gel electrophoresis and transfered to nylon sheets (Hybond N; Amesham Life Science Inc., Arlington Heights, IL) (22). After pre-hybridization for 3 hours, the blots were hybridized with the heat-denatured probe for σ ADH mRNA for 72 hours at 42 °C in a solution containing 50% (v/v) formamide, 5 fold concentrated standard saline phosphate ethylenediaminetetraacetic acid (5X SSPE; 0.75 M sodium chloride, 0.05 M sodium phosphate monobasic, 6.25 mM phosphate ethylene-diaminetetraacetic acid), Denhardt's solution, 0.005% (w/v) sodium pyrophospate, 0.2 mg/ml salmon sperm DNA (Sigma) and 0.1 % sodium dodecylsulfate (SDS; Sigma) (22). The sheets were washed twice with 2X SSC/0.1% SDS for 5 minutes, once with 0.2 X SSC/0.1 % SDS for 5 minutes at room temperature, and once with 0.2 X SSC/0.1 % SDS for 15 minutes at 42 °C (20). Subsequently, they were exposed to X-Omat AR film (Eastman Kodak) for 72 hours at -70 °C using two intensifying screens (Eastman Kodak).

To compare the organ distribution of the *ADH7* gene expression with that of Class I ADH, a probe specific for Class I ADH was prepared; a 439 bp fragment of *ADH1* was amplified by PCR from a human stomach cDNA library (Clontech) with sense (AAGAAACCCTTTTCCATTG) and antisense (AGGCTGCATCAATTT) primers. After stripping the hybridized probe specific for σ ADH with hot water containing 0.5% SDS, the specific probe for Class I ADH labeled with [α-^{32}P] dCTP dCTP (DuPont NEN) was applied to the same blots.

RESULTS

By northern hybridization analysis, mRNA for Class IV ADH was detectable only in the stomach, whereas mRNA for Class I ADH was widely expressed in several organs, particularly in the ileum, lung, liver, and the stomach (Figure 2).

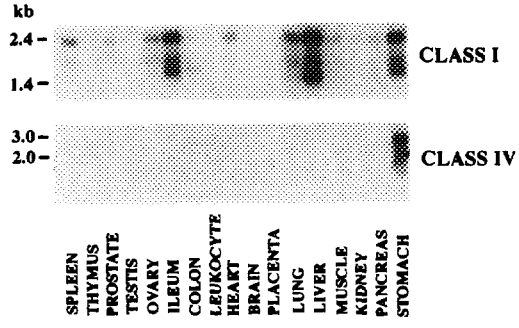

Fig 2. Organ distribution of the expression of Class I and Class IV ADH. The expression of Class I and Class IV ADH were compared by northern hybridization analysis. Class IV ADH was expressed predominantly in the stomach, whereas Class I ADH was extensively expressed in several organs.

The upstream structure of *ADH 7* was examined up to - 537 bp (Figure 3). This region had TATA and CCAAT boxes but no CpG islands. In this region, several consensus sequences of binding sites for transcriptional factors, including metal binding factor-1 (MBF-1), active gene regulatory protein-1 (AP-1), and glucocorticoid response element (GRE) were observed. Only one CCAAT enhancer binding protein (C/EBP) consensus was found. The hepatocyte nuclear factor (HNF) present in the upstream region of *ADH 1* to *3* , was not present.

DISCUSSION

The present northern hybridization analysis demonstrates that *ADH 7* is expressed in the stomach but not in the other organs studied. This organ distribution of mRNA for σADH is in keeping with the finding that σ ADH activity assessed by electrophoresis is found in the upper digestive tract, including the stomach, but not the liver (13,15). It also supports the view that *ADH 7* is not a housekeeping but a tissue specific gene. The upstream region of virtually all housekeeping genes have a CpG island (an extremely GC rich sequence) and lack TATA boxes (23-24), as occurs, for instance, in the upstream region of the ubiquitously

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CTTGCCCTCCTGTTGCAATTATTTTACATTTCCTTGGCTCTGTTTTCATT
   (MBF-1)
TATATTTAATTCCAGAAACCACATCAAGTCTTTGCAGAATGAAGTAGAGC

ATTAAGAAGTAGAGATGTACACACGCATCTCTAAAATCAGCCATGCCTAG
                          (GRE)
 CCAAAGCAGCTTGCACTTAAACACCCAATACATTTTTCATGATTGTGTTG
                        (CCAAT)
AAGTGAAGTAACCTAACCCGTTTTTATATCCTTCAAAATAAGGTGGATAG
                        (TATA)            (C/EBP)
AATGGTTTCAGCCCTTTTCAATAGCTTTGATTATCTTGTTTTTGTTAGAT

CCCTCCTCTTGGTTTGATCATAGTAGTTACTGTATTTCTTTTTATAAGTT
                                                (TATA)
GGTCTGCAAAGGGTAGGGCTTGCAGACCATTGCAAAGTTGTGACGGCTGT
                                                  (AP-1)
GAGTCATATTGCTGAAGGTGGAACTCTGAAGCCAGACTATCTATGTGAAG

GCACAAGCTGCTGTTATATACAACAGAGTGAACTGAGCATCAGTCAGAAA
              (TATA)
AAGTCTATGTTTGCAGAAATACAGATCCAAGACAAAGACAGGatgggc...
                                                MetGly...
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Fig 3. Structure of the upstream region of *ADH7*. The nucleotide sequence of the upstream region of *ADH7* was determined up to -538 b.p. This region had binding sequences for several transcription factors.

expressed *ADH 5* gene (10). The opposite finding in *ADH7* , namely the presence of TATA boxes and the absence of a CpG island, may be related to the restricted organ expression.

We confirmed our previous demonstration that σADH mRNA is not expressed in the human liver (20). C/EBP (CCAAT enhancer binding protein) has been suggested to play a role in liver specific gene transcription (25). Steward et al. (26) have demonstrated that the upstream region of *ADH 1 to 3* , which are strongly expressed in the liver, have multiple C/EBP binding sites. In contrast to these genes, only one C/EBP binding consensus sequence was found in the upstream region of *ADH7*. In addition, the upstream region of *ADH7* lacks HNF (hepatocyte nuclear factor) which has also been implicated in the liver specific expression in *ADH 1* (27). These observations may account for the fact that *ADH7* is not expressed in the liver.

The upstream region of *ADH 7* has consensus sequences of binding sites for several transcriptional factors, including MBF-1 for zinc, AP-1 for c-Fos and c-Jun (28). These factors may contribute to the regulation of the *ADH7* expression.

Duester et al. (29) found that the retinoic acid response element in *ADH 3* upregulates the expression of this gene and suggested that retinoic acid can potentially stimulate its own synthesis via positive feedback, since the isozyme (γ ADH) encoded by this gene is involved in the synthesis of retinoic acid. The Class IV (σ) ADH encoded by *ADH7* has also been considered to be a key enzyme in the metabolism of retinol to retinoic acid (13,17). The retinoic acid response element (AGGTCATGACCT, 34) was not found in the upstream region of *ADH7*. By contrast, retinoic acid could downregulate the latter gene expression via AP-1 (30), which was found in the upstream region of *ADH7* but was not present in the upstream region of *ADH 3* , at least up to -257 b.p. It is, therefore, conceivable that the *ADH7* involvement in the metabolism of retinoic acid may be regulated by a negative feedback mechanism which can keep constant the levels of retinoic acid in organs where this ADH is expressed. Since retinoic acid is necessary for epithelial cell differentiation (31), σADH may be important in the regulation of this process in the upper digestive tract, including the stomach.

Finally, a sequence that was highly compatible with the consensus for GRE (glucocorticoid response element; homology of 11 of 15 nucleotide bases) was found in the upstream region of *ADH7*. A similarly homologous response element in *ADH 2* has been suggested to be

functional (32). Thus, the expression of *ADH 7* gene may also be under the regulation of steroid hormones. In several genes, androgens (but not estrogens) have been found to upregulate the gene expression through GRE (33). Therefore, GRE in *ADH7* may account for the observed gender difference in the first-pass metabolism due to a higher gastric ADH activity in male than in female subjects (34).

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