MOLECULAR CLONING OF THE MOUSE ADH-B_2 CDNA AND THE HUMAN *ADH5/FDH* GENE AND A STUDY ON THE REGULATION OF *ADH5/FDH* GENE EXPRESSION

MAN-WOOK HUR

Submitted to the Faculty of the Graduate School
in Partial Fulfillment of the Requirements
for the Degree
Doctor of Philosophy
in the Department of Biochemistry and Molecular Biology
Indiana University

March, 1993

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

Doctoral Committee

Howard J. Edenberg, Ph.D.
(Committee Chairman)

Peter J. Roach, Ph.D.

William F. Bosron, Ph.D.

Ann Roman, Ph.D.

February 4, 1993

ABSTRACT

Mammalian alcohol dehydrogenases (ADH, EC 1.1.1.1) are zinc-containing dimeric enzymes, which are involved in the reversible oxidation of alcohols to aldehydes. They have been classified according to their enzymatic and electrophoretic properties into class I, class II, and class III. Class III ADHs have unique properties that set them apart from the other ADHs: wide tissue distribution, activity on longer-chain alcohols and ω-hydroxyfatty acids. The class III ADHs are also glutathione-dependent formaldehyde dehydrogenases (FDH, EC 1.2.1.1).

We have cloned and sequenced a cDNA encoding the mouse class III alcohol dehydrogenase, Adh-B_2. Adh-B_2 mRNA is detectable in all the mouse tissues tested, but highest amounts were detected in liver tissue. This mRNA distribution parallels the reported distribution of Adh-B_2 enzyme activity suggesting that this gene is regulated by the steady-state level of its mRNA. Class III ADHs are highly conserved: the deduced amino acid sequence of the mouse Adh-B_2 is 91% to 97% identical to the human, horse and rat liver enzymes. The mouse Adh-B_2 cDNA is 87% identical in nucleotide sequence to the human χ-ADH cDNA.

Also, we have cloned and characterized the human class III alcohol dehydrogenase gene, *ADH5/FDH*, which encodes χ-ADH. Two overlapping genomic clones (λC-4 and λ7-1) cover the entire *ADH5/FDH*. These clones were characterized by restriction mapping, extensive sequencing and primer extension. The *ADH5/FDH* gene is 15 kb in size and is composed of 9 exons and 8 introns. The introns are located in the same positions as found for the class I and class II ADH genes, which firmly places *ADH5/FDH* as a member of the ADH gene family. The sizes of the introns do not match the other ADH genes; in particular introns 5, 6, and 8 are much smaller than in the class I genes. The 5' end is very GC rich (75% in 150 bp upstream from the ATG codon). It contains GC boxes, but no TATA and CCAAT boxes. Interestingly, one of the χ-ADH cDNAs was reported to contain a sequence

for a 19 aa leader polypeptide; no such leader polypeptide was present in the reported protein sequence. The particular upstream translation initiation codon for this leader was not observed in the nucleotide sequence of the *ADH5/FDH* nor did we find it in our sequencing of the cDNA. We also isolated several related pseudogenes of *ADH5/FDH*, both processed and partially processed. These pseudogenes were mapped to chromosomes 1, 5, and 6, whereas *ADH5/FDH* gene is on chromosome 4.

We further characterized the 5' upstream region of *ADH5/FDH* by CAT assays, DNase I footprinting assays, and gel retardation assays. Five regions in the *ADH5* promoter (-464 bp to -164 bp; -163 bp to -128 bp; -127 bp to -65 bp; -64 bp to -35 bp; -34 to +61 bp) contained cis-acting elements important in the regulation of *ADH5/FDH* expression. The small region from -34 bp to +61 bp showed strong promoter activity in all three cell lines. The contributions of the positive cis elements in the region from -163 bp to -128 bp and from -64 bp to -35 bp and a negative element in the region from -127 bp to -65 bp were distinctively different in three different cell lines. A region footprinted by all nuclei extracts and Sp1 protein (-38 bp to +22 bp) may explain the basal level of expression of *ADH5/FDH*. In part, the level of transcription factors that interact with the elements located upstream of -110 bp may be critical factors in the higher level of expression in tissues such as liver and kidney.

Doctoral Committee

Howard J. Edenberg, Ph.D.
(Committee Chairman)

Peter J. Roach, Ph.D.

William F. Bosron. Ph.D.

Ann Roman, Ph.D.

February 4, 1992

TABLE OF CONTENTS