[*Chem.-Biol. Interact.*, **130**, 775-784 (2001)] [Lab. of Biochemistry]

Identity of Dimeric Dihydrodiol Dehydrogenase as NADP$^+$-dependent D-Xylose Dehydrogenase in Pig Liver.

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Dimeric dihydrodiol dehydrogenases (DDs), which oxidize *trans*-dihydrodiols of aromatic hydrocarbons to the corresponding catechols, have been molecularly cloned from human intestine, monkey kidney, pig liver, dog liver, and rabbit lens. A comparison of the sequences with the DNA sequences in databases suggested that dimeric DDs constitute a novel protein family with 20 gene products. In addition, it was found that dimeric DD oxidizes several pentoses and hexoses, and the specificity resembles that of NADP$^+$-dependent D-xylose dehydrogenase of pig liver. The inhibition of D-xylose dehydrogenase activity in the extracts of monkey kidney, dog liver and pig liver, its co-purification with dimeric DD activity from pig liver, and kinetic analysis of the D-xylose dehydrogenation by pig dimeric DD indicated that the two enzymes are the same protein.

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Crystallization and Preliminary X-ray Diffraction Analysis of Monkey Dimeric Dihydrodiol Dehydrogenase.

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Dihydrodiol dehydrogenase catalyzes the NADP$^+$-linked oxidation of *trans*-dihydrodiols of aromatic hydrocarbons to corresponding catechols and exists in multiple forms in mammalian tissues. The dimeric form of mammalian dihydrodiol dehydrogenase has a primary structure distinct from the previously known mammalian enzymes and may constitute a novel protein family with the prokaryotic proteins. Monkey kidney dimeric dihydrodiol dehydrogenase was crystallized from buffered ammonium phosphate solution using the hanging drop vapour-diffusion method. The crystals diffract to 2.65 Å resolution in the laboratory and belong to hexagonal $P6_1$, $P6_5$, $P6_122$ or $P6_522$ space group with $a=b=122.8$, $c=121.3$ Å, $\alpha=\beta=90°$ and $\gamma=120°$.

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Enzymatic Characteristics and Subcellular Distribution of A Short-Chain Dehydrogenase/Reductase Family Protein, P26h, in Hamster Testis and Epididymis.

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A hamster sperm 26-kDa protein (P26h) shows striking homology with mouse lung carbonyl reductase (MLCR[1]) and is highly expressed in the testis, but its physiological functions in the testis are unknown. We show that recombinant P26h resembles NADP(H)-dependent MLCR in the tetrameric structure, broad substrate specificity, inhibitor sensitivity and activation by arachidonic acid, but differs in a preference for NAD(H) and high efficiency for the oxidoreduction between 5α-androstane-3α,17β-diol (k_{cat}/K_M=243 $s^{-1}mM^{-1}$) and 5α-dihydrotestosterone (k_{cat}/K_M=377 $s^{-1}mM^{-1}$). These results suggest that P26h mainly exists as a tetrameric dehydrogenase in mitochondria of testicular cells, and plays a role in controlling the intracellular concentration of a potent androgen, 5α-dihydrotestosterone, during spermatogenesis, in which it may be incorporated in mitochondrial sheaths of spermatozoa.

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Co-operative Regulation of the Transcription of Human Dihydrodiol Dehydrogenase (*DD*)*4*/Aldo-Keto Reductase (*AKR*)*1C4* Gene by Hepatocyte Nuclear Factor (HNF)-4α/γ and HNF-1α.

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Human dihydrodiol dehydrogenase (DD) 4/aldo-keto reductase (AKR) 1C4 is a major isoform of hepatic DD that oxidizes *trans*-dihydrodiols of polycyclic aromatic hydrocarbons to reactive and redox-active *o*-quinones and that reduces several ketone-containing drugs. To investigate the mechanism of transcriptional regulation of the human *DD4* gene, the 5'-flanking region of the gene was fused to the luciferase gene. The formation of the DNA-protein complex was inhibited by the HNF-4 or HNF-1 motif of the α_1-antitrypsin gene. A supershift assay using antibodies to human HNF-4α, HNF-4γ and HNF-1α showed that HNF-4α and HNF-4γ bound to the HNF-4 motif, and that HNF-1α interacted with the HNF-1 motif. Introduction of mutations into the HNF-4 or HNF-1 motif lowered the luciferase activity to 10 or 8% respectively of that seen with the intact human *DD4* gene. These results indicate that HNF-4α, HNF-4γ and HNF-1α regulate co-operatively the transcription of the human *DD4* gene in HepG2 cells.