〔Biochem. Biophys. Res. Commun., **145**, 1260 (1987)〕

Purification and Partial Characterization of Dimeric Dihyrodiol Dehydrogenase from Monkey Kidney.

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Dihydrodiol dehydrogenase activity was detected in the cytosol of several monkey tissues, among which kidney exhibited the highest activity and contained a high-molecular weight (Mr 65,000) enzyme species. The enzyme species was purified to apparent homogeneity and showed a subunit molecular weight of 39,000. The enzyme oxidized benzene dihydrodiol (Km=0.9 mM) at a pH optimum of 9.8, and reduced vicinal diketones such as camphorquinone (Km=0.1 mM) and diacetyl (Km=0.8 mM) around pH 7.5, but alicyclic alcohols, hydroxysteroids and ketosteroids were inactive substrates for this enzyme. Quercitrin, SH-reagents, stilbestrol were inhibitory to the enzyme activity, but other synthetic estrogens, anti-inflammatory agents and 3-ketosteroids were not.

〔Biochim. Biophys. Acta, **912**, 270 (1987)〕

Purification and Properties of Two Multiple Forms of Dihydrodiol Dehydrogenase from Guinea-Pig Testis.

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Dihydrodiol dehydrogenase activity in the cytosol of guinea-pig testis was separated into two major and two minor peaks by Q-Sepaharose chromatography. The two major enzyme forms were purified to homogeneity. One form with a Mr 32,000 and pI 4.2, which had the highest amount in the tissue, showed strict specificity for benzene dihydrodiol and NADP, and reduced pyridine aldehydes and diacetyl at low rates. Another form, with a Mr 36,000 and pI 5.0, oxidized n-butanol, glycerol and sorbitol as well as benzene dihydrodiol in the presence of NADP or NAD, and exhibited much higher reductase activity on various aldehydes, aldoses and diacetyl. The pI 5.0 form was inhibited potently by sorbinil and was activated by sulfate ion.

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Kinetic and Stereochemical Studies on Reaction Mechanism of Mouse Liver 17β-Hydroxysteroid Dehydrogenases.

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The kinetic mechanism of two major monomeric 17β-hydroxysteroid dehydrogenases from mouse liver cytosol was studied at pH 7 in both directions with NADP(H) and three steroid substrates. Initial velocity analyses and inhibition patterns by products and dead-end inhibitors were consistent with an ordered bi bi mechanism with the coenzyme binding to the free enzyme. Binding studies of the coenzyme and substrate indicate that 1 mol of coenzyme binds to 1 mol of each enzyme. The 4-pro-R-hydrogen atom of NADPH was transferred to the α-face of the steroid molecule by the two enzymes.