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Kinetic Alteration of Human Dihydrodiol/3α-Hydroxysteroid Dehydrogenase Isoenzyme , AKR1C4, by Replacement of Histidine-216 with Tyrosine or Phenylalanine.

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Human dihydrodiol dehydrogenase with 3α-hydroxysteroid dehydrogenase activity exists in four forms (AKR1C1-1C4) that belong to the aldo-keto reductase (AKR) family. Recent crystallographic studies on the other proteins of this family have indicated a role of a tyrosine residue (corresponding to position 216 in these isoenzymes) in stacking the nicotinamide ring of the coenzyme. The mutant enzymes of AKR1C4 (H216Y and H216F) decreased the K_m for $NADP^+$ by 3-fold, and differently influenced the K_m and k_{cat} for substrates, the sensitivity to competitive inhibitors. Furthermore, the mutation decreased the stimulatory effects of the enzyme activity by sulphobromophthalein, clofibric acid and thyroxine. These results indicate the importance of this histidine creating the cavity of the substrate-binding site of AKR1C4 through the orientation of the nicotinamide ring of the coenzyme as well as its involvement in the conformational change by binding non-essential activators.

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Roles of His-79 and Tyr-180 of D-Xylose/Dihydrodiol Dehydrogenase in Catalytic Function.

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Mammalian dimeric dihydrodiol dehydrogenase is identical with D-xylose dehydrogenase, and belongs to a protein family with prokaryotic proteins including glucose-fructose oxidoreductase. Of the conserved residues in this family, either His-79 or Tyr-180 of D-xylose/dihydrodiol dehydrogenase has been proposed to be involved in the catalytic function. A mutant, Y180F, was almost inactive, but, similarly to the wild-type enzyme, exhibited high affinity for NADP(H) and fluorescence energy transfer upon binding of NADPH. The H79Q mutation had kinetically largest effects on K_d (>7-fold increase) and K_m (>25-fold increase) for NADP(H), and eliminated the fluorescence energy transfer. Interestingly, the dehydrogenase activity of this mutant was potently inhibited with a 190-fold increase in the K_m for $NADP^+$ by high ionic strength, which activated the activity of the wild-type enzyme. These results suggest a critical role of Tyr-180 in the catalytic function of this class of enzymes, in addition to functions of His-79 in the coenzyme binding and chemical steps of the reaction.

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Structure-specific Effects of Thyroxine Analogs on Human Liver 3α-Hydroxysteroid Dehydrogenase.

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A major isoform of human liver 3α-hydroxysteroid dehydrogenase is a unique member of the aldo-keto reductase family, which is activated by several drugs such as bromosulfophthalein, clofibric acid derivatives and anti-inflammatory 2-arylpropionic acids. Here, we investigated the endogenous activator for this enzyme, and found that D-thyroxine (T_4), L-T_4 and DL-reverse T_3 were non-essential activators which showed maximal stimulation of 5-, 4- and 2-fold, respectively, and the respective K_A values of 1.5, 1.1 and 3.6 μM. L-T_3, DL-thyronine and D-tyrosine had no effect on the enzyme, but 3,5,3',5'-tetra- and 3,5,3'-triiodothyropropionic acids were potent competitive inhibitors of K_i values of 42 and 60 nM, respectively, with respect to the substrate. These results indicate that, depending on their structures, the T_4 analogs bind differently to two distinct sites at the active center of the enzyme to produce stimulatory and inhibitory effects.

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Catalytic Properties for Naphthoquinones and Partial Primary Structure of Rabbit Heart Acetohexamide Reductase.

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The rabbit heart acetohexamide reductase (RHAR) efficiently reduced 1,4-naphthoquinone and juglone, whereas it had little or no ability to reduce menadione or plumbagin. The structural requirements for these four naphthoquinones and one acetohexamide analog, and the kinetic mechanism for the inhibition of acetohexamide reduction by juglone led us to conclude that the 2-methyl group of menadione and plumbagin prevents access of the substrates to the catalytic site of RHAR. Five of six peptides derived from RHAR showed 30-42% residue identities with regions in the amino acid sequence of mouse lung carbonyl reductase (MLCR) belonging to the short-chain dehydrogenase/reductase (SDR) family. The catalytically important residues (Arg-39, Ser-136, Tyr-149 and Lys-153) of MLCR were found in the peptide sequences of RHAR, despite the low residue identities between the two enzymes. RHAR is probably best classified as a member of the SDR family similar to MLCR.