3D Model of Ternary Complex of Human 3β-HSD type I. Rational Mutagenesis

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Mammalian 3β-Hydroxysteroid Dehydrogenase/Isomerase (3β-HSD) catalyzes conversion of dehydroepiandrosterone and pregnenolone to active hormones, progesterone and androstenedione. A 3D model of ternary complex of human 3β-HSD type I complexed with NAD cofactor and androstenedione product has been developed based upon two X-ray structures, the UDP-galactose 4-epimerase (UDPGE) complexed with an NAD cofactor and substrate, and the 17β-hydroxysteroid dehydrogenase (17β-HSD) complexed with an NADP cofactor and the androstenedione substrate. These enzymes share 21% and 15% sequence identity with 3β-HSD_1 enzyme in the overlapping regions. The cofactor and substrate binding sites in 3β-HSD_1 resemble the corresponding sites in UDPGE and 17β-HSD structures. A dimer structure of 3β-HSD_1 with a stereochemically optimal interface was built by respective 3D superposition with both subunits of dimeric structure of DTDP-D-glucose 4,6-dehydratase with which 3β-HSD shares 19% sequence identity. The 3D structure of 3β-HSD enzyme is in good agreement with existing biochemical data and is being used to design rational mutations to elucidate key substrate binding residues in the active site and the basis for enzyme dual oxidoreductase and isomerase functions. As predicted by the 3D model, mutagenic data have confirmed a role for H232 in recognizing the 17-keto group of the bound substrate. The H232A mutant lacks the oxidoreductase activity but retains the isomerase activity.

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