Characterization of Baboon 3β-Hydroxysteroid Dehydrogenase

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The 3β-hydroxysteroid dehydrogenase (3β-HSD)/isomerase enzymatic system plays an important role in the biosynthesis of all classes of hormonal steroids. Human 3β-HSD catalyses the oxidative conversion of 3β-hydroxy-5-ene-steroids (pregnenolone and dehydroepiandrosterone) to the corresponding 3-oxo-4-ene-steroids (progesterone and androstenedione) in a two-step sequential reaction - a dehydrogenase activity that is NAD^+-dependent, followed by an isomerase activity. NADH formed induces a conformational change in the enzyme, activating the isomerase activity. In other species 3β-HSD isoforms display a dual functionality - mouse and rat 3β-HSD exhibit a dehydrogenase activity as well as a NADPH dependent 3-ketosteroid reductase activity.

Characterization of 3β-HSD isoenzymes identified two distinct genes in humans encoding type 1 in the placenta, endometrium, mammary gland and skin, and type 2 in the adrenals and gonads. The sequence spans 8 kb and includes 4 exons and 3 introns. The cDNA is 1122bp and encodes 374 amino acids. The 3β-HSD activity resides within a single dimeric protein with a relative molecular mass of 84 kDa.

Cape Baboon (*Papio Ursinus*) 3β-HSD cDNA was prepared from adrenal cortex tissue and shows 93% and 95% amino acid homology with the human type 1 and 2, respectively. Four recombinant constructs have been cloned, analysed and expressed in nonsteroidogenic mammalian COS-1 cells to determine their catalytic activity. The influence of the amino acid substitutions on the apparent K_m of the constructs will be presented.