Supplemental Data File

Biochemical Factors Governing the Steady-State Estrone/Estradiol Ratios Catalyzed by Human 17β-Hydroxysteroid Dehydrogenases Types 1 and 2 in HEK-293 Cells

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Supplemental Methods

Intrinsic rates of half-reactions

Concentrations of E1 and E2 were obtained from pilot experiments using tritium-labeled steroids in the stable cell line, which gave lower equilibrium proportions of E2 than found in transfected HEK-293 cells (80% E2, 20% E1 in complete medium; 60% E2. 40% E1 in 2-deoxyglucose). Next, [^{14}C]-labeled E2 is added to give approximately 500,000 CPM, which is enough to provide reliable signal-to-noise for the requisite number of samples taken. From this, the concentration of E2 is calculated using the specific activity. Finally, an amount of unlabeled E1 is added to yield the correct concentration ratios, along with 600,000-1,000,000 CPM of [^{3}H]-labeled E1, which also is sufficient to provide adequate counting statistics. Note that the tritium-labeled steroid has negligible mass and does not contribute to the mass calculations.

Initial rates are determined by taking the first derivatives of the curves and setting t = 0: $f(t) = A \bullet e^{-kt} + C$, the first derivative is $f'(0) = A \bullet k$. The rate decreases over time to 0 at $t = \infty$.

Supplemental Discussion

Complexity of cofactor binding by 17βHSD1

Four amino acids contribute predominantly to cofactor binding by 17βHSD1: S13 (in the conserved GXXXGXG motif), R38, D66, and K196. To understand the structural changes that might influence cofactor affinity, we show the binding pocket in its 3 possible states in Supplemental Data Figure 4: Panel A (cyan) represents the apo-enzyme, Panel B (green) NAD+ bound form, Panel C (salmon) NADP+ bound form. The bottom Panels D and E represent the superimposition of the 3 structures and the superimposition of the cofactors, respectively, as extracted from their original structures (PDB access codes: 1iol apo, 1fdv with NAD+, and 1equ with NADP+). One of the major transformations, which occur when 17βHSD1 binds NAD+, is that the small helix containing K196 transitions to a loop. Within 3 Å of the cofactor, S13, D66, and K196, establish electrostatic interactions, while R38 is 4 Å from NAD+.

When the cofactor-binding pocket is occupied with NADP+, R38 is crucial for the interactions with the 2'-phosphate group of NADP+. S13 is found also within 3 Å of the cofactor. On the other hand, K196 drastically changes orientation, facing opposite to the binding pocket, while D66 moves 5 Å away from the point of contact seen in the NAD+-bound structure (Panels B and C). The change in D66 is due to a rotation of the side chain, since superimposition of the structures (Panel D) demonstrates that there is little or no movement of this loop.

When cofactors are superimposed (Panel E), slightly different conformations are found, despite great similarities in their structures. Since major structural movement is observed only in the segment of 17βHSD1 containing K196, we conclude that the flexibility in both cofactor and residue side chains in the vicinity of the cofactor-binding pocket participate in cofactor binding.