Influence of Cellular Localization on Activity of Hydroxysteroid Dehydrogenases

Kerry Michael Wooding, Ph.D.
The University of Texas Southwestern Medical Center at Dallas, 2011
Graduate School of Biomedical Sciences
Supervising Professor: Richard J. Auchus, M.D. Ph.D.

Date Available: 12/12/2013
Summer 2011
Biological Chemistry
Bibliography pp. 107-117

Keywords: 17 β -HSD1 (17 Beta Hydroxysteroid Dehydrogenase Type 1); AKR1C9 (Aldo-Keto Reductase Type 1C9); 11 β -HSD1 (11 Beta Hydrozysteroid Dehydrogenase Type 1); H6PD (Hexose-6-Phosphate Dehydrogenase); cellular localization

Hydroxysteroid dehydrogenases (HSDs) catalyze the interconversion of inactive steroids and active hormones. HSDs use nicotinamide cofactors in the cytosol and endoplasmic reticulum (ER) lumen to either reduce or oxidize their steroid substrates. Our lab has extensively studied the 17beta-HSDs types 1, 2 and 3 of the short-chain oxidoreductase family, particularly human 17beta-HSD1, which favors estrone reduction to estradiol. Rat AKR1C9 has also been thoroughly studied as a model HSD of the aldo-keto reductase family; AKR1C9 catalyzes the reduction of dihydrotestosterone to androstanediol. These two enzymes provide a basis for comparative studies with 11beta-HSD1, which catalyzes the reduction of cortisone to cortisol.

Most mammalian cells supplied with adequate glucose and oxygen maintain cytoplasmic high nicotinamide concentration gradients, [NADPH] >> [NADP+] and [NAD+] >> [NADH], and in the strongly oxidizing environment of the ER lumen, both these gradients are shifted to more oxidized cofactor. Whereas 17betaHSD types 1, 2, 3 and AKR1C9 catalyze their respective reactions in a thermodynamically predictable manner based on cofactor gradients, 11beta-HSD1 does the opposite. 17beta-HSD1, 17beta-HSD3, and AKR1C9 favor reduction in the cytosol using NADPH, and 17beta-HSD2 favors oxidation in the ER lumen using NAD+. In contrast, 11beta-HSD1 reduces cortisone to cortisol in the highly oxidative ER lumen but requires hexose-6-phosphate-dehydrogenase (H6PD) to regenerate NADPH in the ER lumen. We hypothesize that H6PD directly channels NADPH to 11beta-HSD1 through specific interactions.

To test this hypothesis, we have targeted 17beta-HSD1 and AKR1C9 to the ER lumen rather than the cytosol. Conversely, we have targeted 11beta-HSD1 to the cytoplasmic surface of the ER. In addition, we have engineered point mutations in 17beta-HSD1, AKR1C9, 11beta-HSD1 and H6PD, designed to attenuate the directional preferences by altering cofactor binding. Targeting and retaining 17beta-HSD1 in the ER lumen proved troublesome; regardless of the transfection conditions, ER-targeted 17beta-HSD1 was always detected in the cytosol. We conclude that either 17beta-HSD1's activity or structure causes its translocation to the cytosol.