

The effects of mutating Tyr9 and Arg15 on the structure, stability, conformational dynamics and mechanism of GSTA3-3

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ABSTRACT:

Glutathione S-transferase A3-3 is the most catalytically efficient steroid isomerase enzyme known in humans, transforming Δ^5 -androstene-3-17-dione into Δ^4 -androstene-3-17-dione. GSTA3-3 catalyzes this reaction with ten-fold greater efficiency than GSTA1-1, its closest competitor in the Alpha class of GSTs. In order to examine the differences between Alpha class GSTs and to better elucidate the mechanism of GSTA3-3 the roles of Tyr9 and Arg15 were examined. Tyr9 is the major catalytic residue of Alpha class GSTs and Arg15 is proposed to be catalytically important to GSTA3-3 but never before experimentally examined. While the structure and stability of the Alpha class enzymes are highly comparable, subtle differences at the G-site of the enzymes account for GSTA3-3 having a ten-fold greater affinity for the substrate GSH. Y9F and R15L mutations, singly or together, have no effect on the structure and stability of GSTA3-3 (the same effect they have on GSTA1-1) despite the R15L mutation removing an interdomain salt-bridge at the active site. Hydrogen-deuterium exchange mass spectrometry also revealed that neither mutation had a significant effect on the conformational dynamics of GSTA3-3. The R15L and Y9F mutations are equally important to the specific activity of the steroid isomerase reaction; however, Arg15 is more important for lowering the pK_a of GSH. Lowering the pK_a of GSH being how GSTs catalyze their reactions. Additionally, there is evidence to suggest that Arg15 is integral to allowing GSTA3-3 to differentiate between Δ^5 -androstene-3-17-dione and Δ^4 -androstene-3-17-dione, indicating that Arg15 is a more important active-site residue than previously known.