General Base Positioning and Catalytic Rates: Mechanistic Insight on The Effects of Point Mutations in Δ^5-3 Ketosteroid Isomerase

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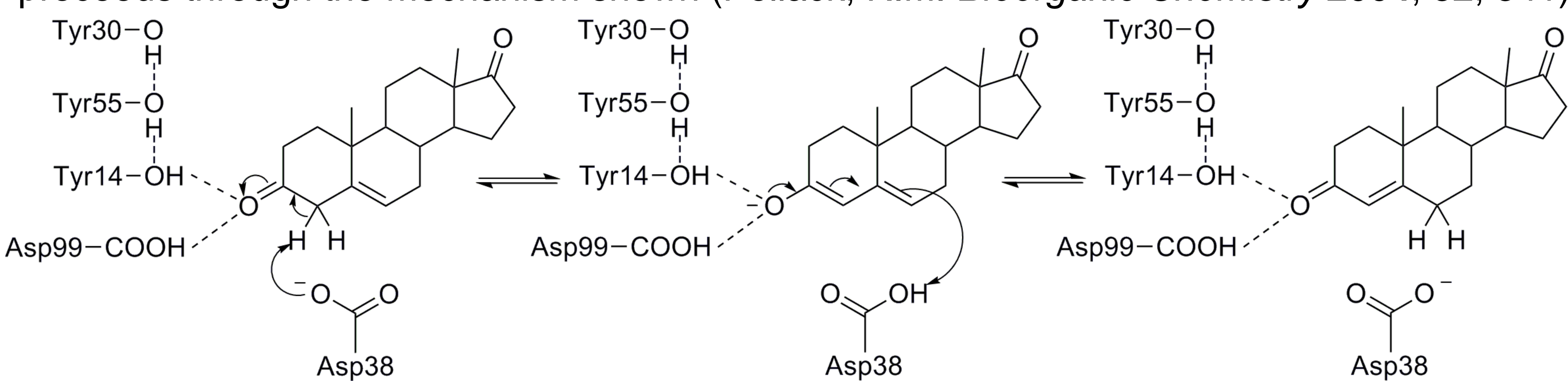
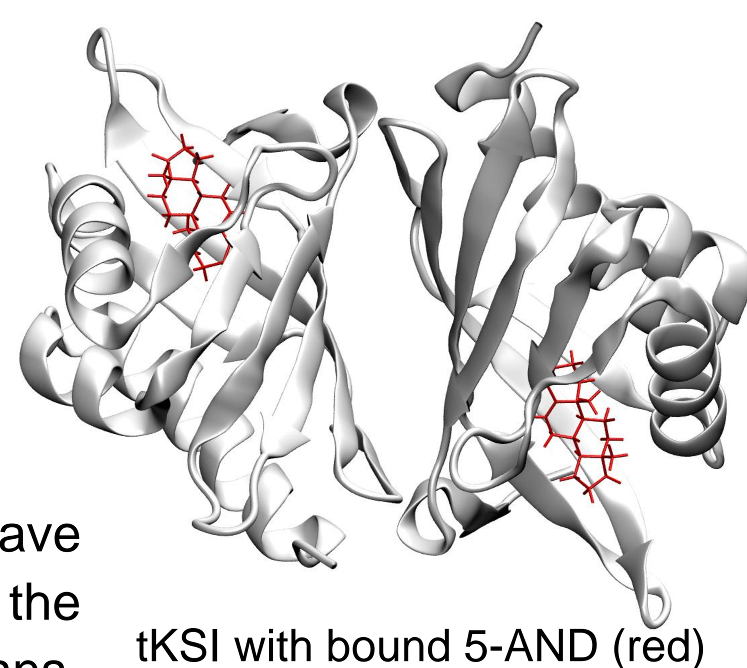
Background

Δ^5-3 ketosteroid isomerase (KSI) is an enzyme used by bacteria in steroid biosynthesis, and proceeds through the mechanism shown (Pollack, R.M. *Bioorganic Chemistry* **2004**, 32, 341):



In this study, wild-type (WT) *Psuedomonas testosteroni* KSI (tKSI) and three tKSI mutants were studied. The D38E mutant was expected to have steric inhibitions that forced the catalytic base away from optimum conformation. The P39G/V40G/S42G mutant, for simplicity referred to as the "P39G" mutant, was hypothesized to have increased flexibility in the active site loop due to the poly-glycine mutation. The "D38E/P39G" mutant had all of the above mutations.



tKSI with bound 5-AND (red)

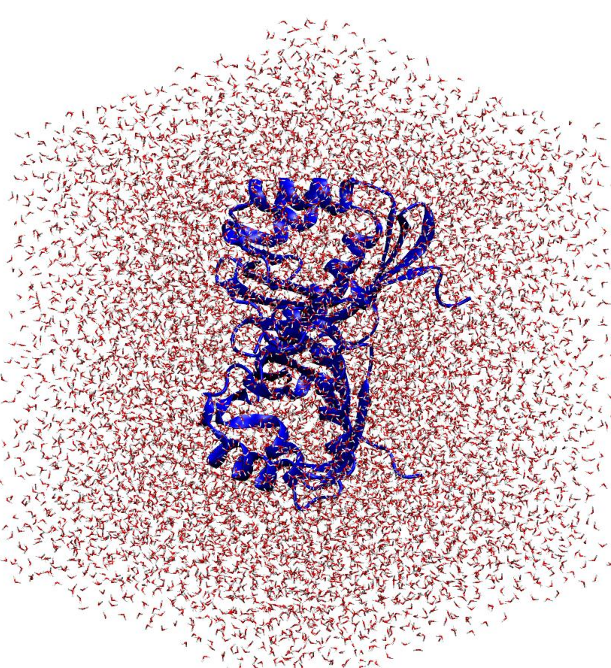
Experimentally, the D38E and P39G mutations were found to have similarly negative effects on the catalytic rate, but the rate of the D38E/P39G mutant is only slightly less than that of either (Schwans, J. and Herschlag, D. Manuscript in preparation). This suggests that the D38E and P39G mutations act non-independently.

Purpose

To determine whether tKSI positions Asp38 in a rigid position within the active site, and to investigate the effect on positioning and molecular mechanism involved in the catalytic rate reductions in the D38E and P39G mutations in tKSI.

Methods

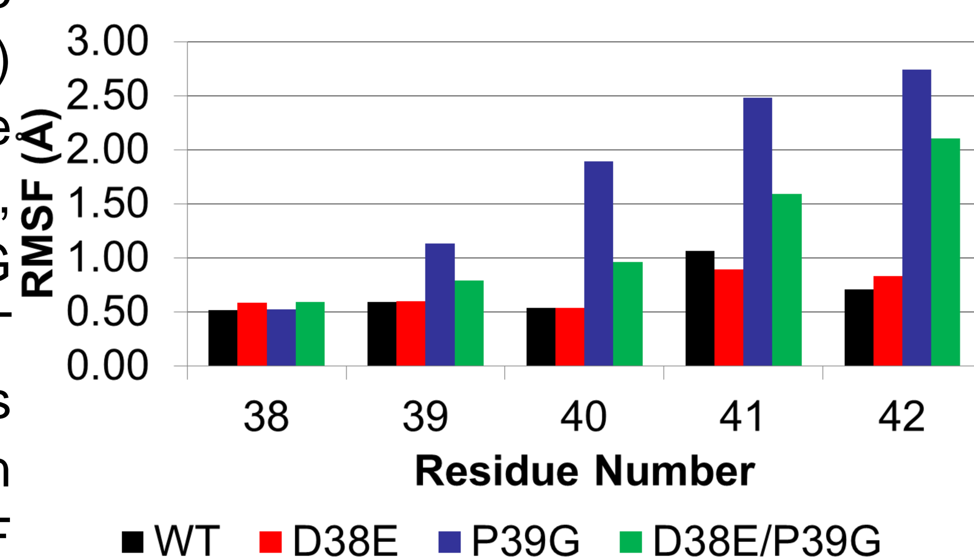
Starting from x-ray crystal structures, WT and the mutants were computationally modeled with bound 5-AND intermediate. After solvating each enzyme in water and Na^+ counterions, standard minimization and equilibration procedures were used to prepare simulations at 300 K and a volume consistent with a pressure of 1 atm. Using the AMBER99SB force field parameters for protein and TIP3P model for water, each system was then simulated for 20 ns. Donor-acceptor distances and positions were recorded to quantify the active sites, while root mean square fluctuation plots were used to measure the flexibility of each region of protein. Additionally, atomic isodensity surfaces were calculated for the catalytic base oxygen and the proton.



Solvated tKSI

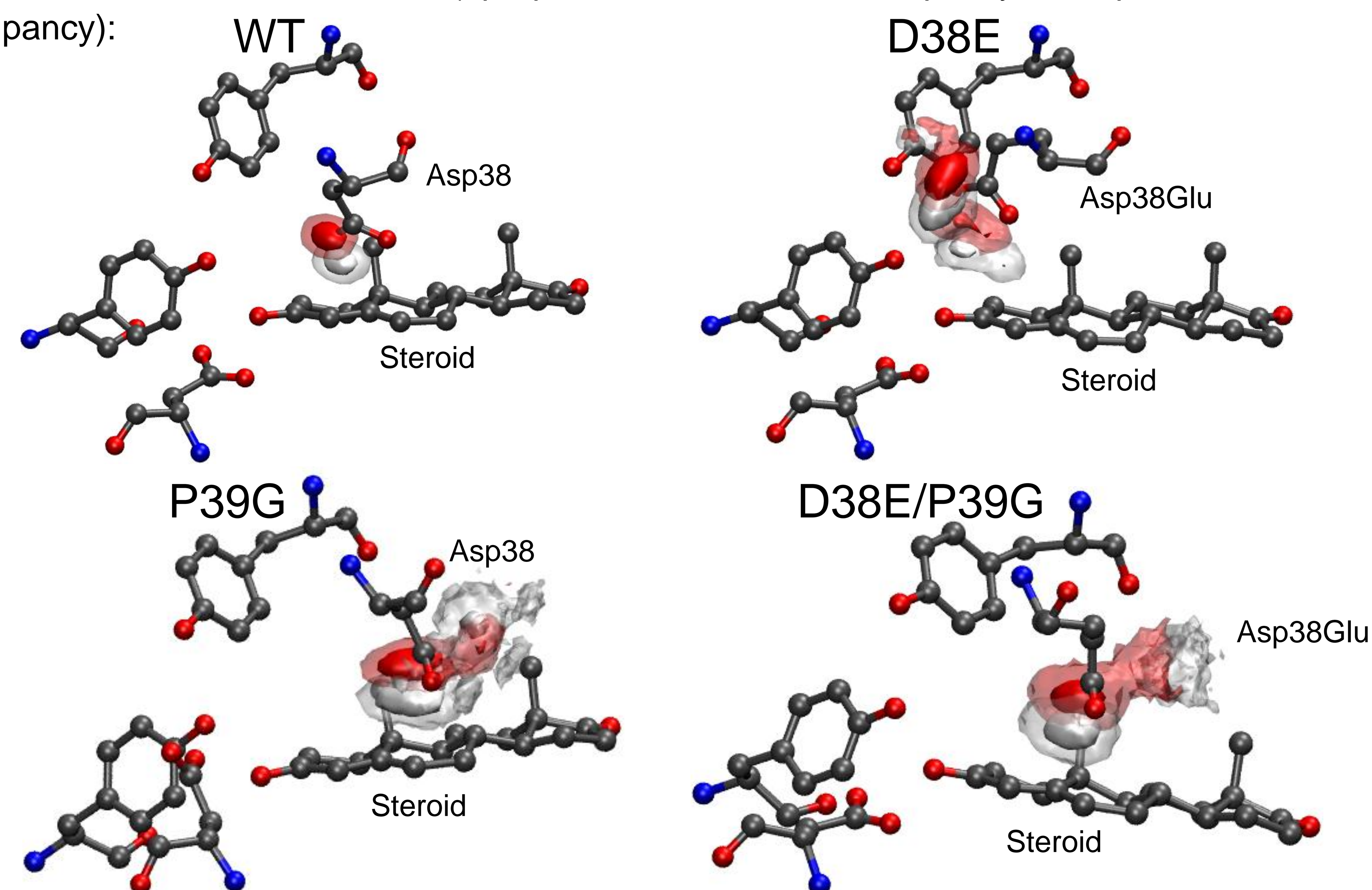
Results

To quantify flexibility of the protein backbone, we measured the C_α root mean square fluctuation (RMSF) for each protein residue in the loop region by the active site. The P39G mutant has the highest RMSF values, indicating that it has the most flexibility. The D38E/P39G mutant has the next-highest RMSF values, while the WT and D38E mutant have similar RMSF values. This supports the hypothesis that the P39G mutation increases backbone flexibility. Interestingly, the RMSF values for residue 38 are quite similar for each system.

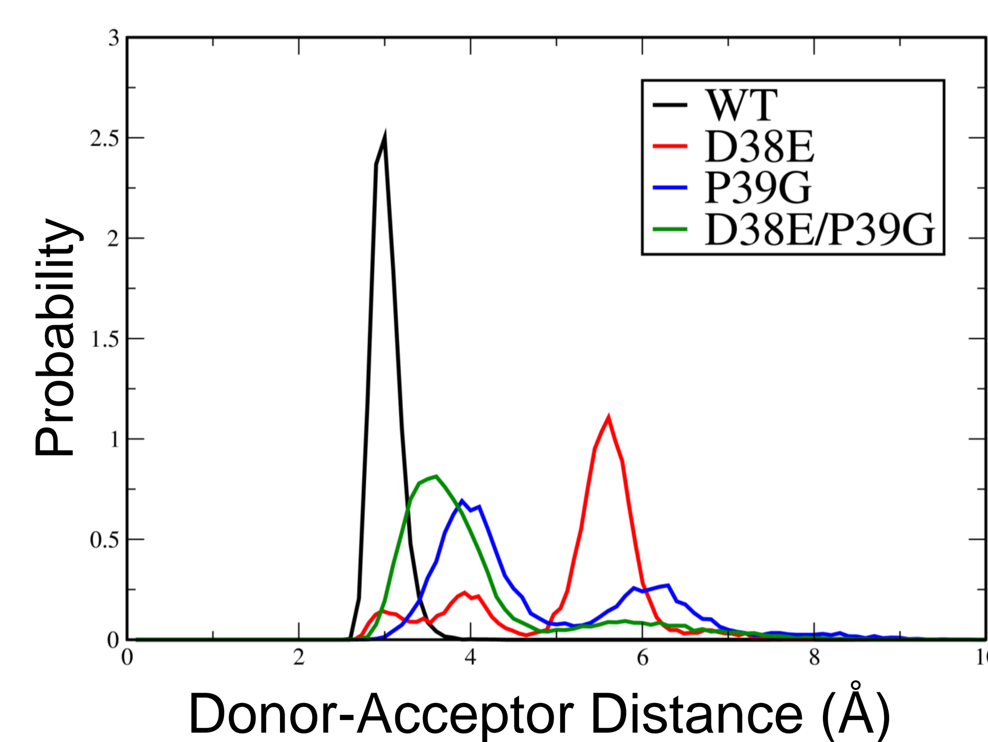


Results (cont.)

Representative isodensity surface plots of the proton donor oxygen (red) and proton (white) for the intermediate of the KSI reaction (opaque surface at 50% occupancy, transparent surface at 90% occupancy):



In the WT isodensity surface, both the proton donor and proton are relatively localized in a productive conformation. This suggests that tKSI does hold the general base in a fairly rigid productive conformer. In the D38E mutant, however, the proton donor and proton appear to substantially populate an unproductive conformation, consistent with the rate reduction observed. The general base in the P39G mutant has a dispersed density. The D38E/P39G mutant also exhibits considerable mobility.



The distance between the hydrogen donor and acceptor was plotted as a histogram for each system. WT has a large peak at 3.0 Å, suggesting it is most frequently aligned in the productive conformation. The D38E mutant has a significant peak at 5.8 Å, suggesting a largely populated unproductive conformation. The P39G mutant has the broadest peak, supporting the hypothesis that its increased flexibility results in a less populated productive conformation. The D38E/P39G histogram is similar to that of the P39G mutant, suggesting that increased flexibility of the poly-glycine mutation may overwhelm the steric effects of the D38E mutation.

Conclusions

Our results support the hypothesis that WT tKSI does position the general base in a rigid position within the active site. D38E and P39G mutations disrupt this positioning, and the hypotheses that the D38E mutant adopts an unproductive conformation while the P39G mutant has a less populated productive conformation are supported. The non-additivity exhibited by the D38E/P39G mutant seems to be due to the increased flexibility of the active site loop overshadowing the steric effects of the D38E mutation.

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