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Understanding the Role of Loop-Loop Interactions Through Mutagenesis Studies on Triosephosphate Isomerase

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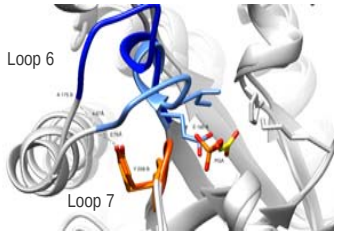
ABSTRACT

Triosephosphate isomerase (TIM) utilizes desolvation of the active site and electrostatic interactions to efficiently catalyze the isomerization reactions between D-glyceraldehyde-3-phosphate (D-GAP) and dihydroxyacetone phosphate (DHAP). Upon substrate binding, TIM's loop 6 closes and situates the catalytic base towards the substrate for proton abstraction. Loop 6 is stabilized in the closed conformation through interactions with loop 7.Inhibition studies with 3-phosphoglyycolic acid (PGA), a transition state analog, were conducted on Y208 mutants to elucidate its role in stabilizing the close, active conformation. The results suggest that the tyrosine residue is involved in the facilitation of loop-6/7 hydrogen bonding. TheY208T mutation had little effect on the pK_a of the active catalytic base, E165. Y208A and Y208F mutants perturbed the pKa of E165, resulting in values of 9.0 and 7.5 respectively.

INTRODUCTION

The thermodynamically favorable reaction of TIM with D-GAP to form DHAP through an endiolate intermediate.

Triosephosphate isomerase (TIM), a glycolytic enzyme, catalyzes the inter-conversion of dihydroxyacetone phosphate (DHAP) to D-glyceraldehyde phosphate (D-GAP) via an *cis*-endiolate intermediate. TIM catalyzes the reaction by abstracting a proton from the $C_{1,}$ carbon creating the intermediate and the donation of the proton to the C_2 carbon to form the product.[1]

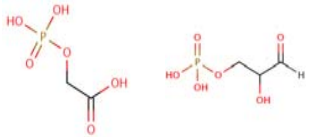


Superimposed active site of free enzyme (dark blue/orange) and closed conformation (light blue/orange) while bound to transition state analog phosphoglycolic acid (PGA).

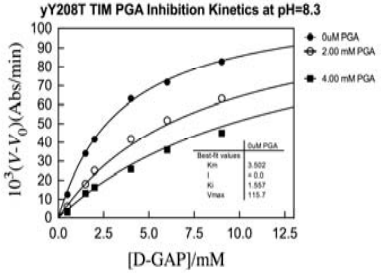
TIM is considered a " perfect enzyme"indicating that its rate of reaction approaches the rate of a diffusion-controlled reaction.[2] Past experiments have indicated that the second order rate constant (k_{cat}/K_m) for the isomerization of D-GAP to DHAP is $10^{7.7}$ times faster than the free base catalyzed reaction in water, even while the reaction pathway is conserved.[3] Additionally at least 80% of the catalysis is due to the intrinsic bond energy of the phosphodianion, which coordinates the enzyme and substrate for the close conformation and catalysis.[4]

INHIBITION ASSAYS

The pK_a of the catalytic glutamic acid (E165) was examined by determining the pH dependence of inhibition constants for the competitive inhibitor 3-phospoglycolic acid (PGA), a transition state analog. Inhibition assays were conducted at pH's varying from 4.9 to 9.3 at an ionic strength of 0.1 and at 25ºC. Mutations were made to the Y208 residue to study the effects of perturbing the loop 6/7 hydrogen bonding.



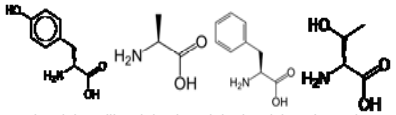
Structures of PGA (left) and D-GAP (right).



Inhibition curve, modeled by Michaelis-Menten inhibition kinetics, of Y208T TIM at a pH of 8.3. Velocities are corrected by the background reaction of D-GAP to DHAP.

It has been shown that PGA trianion binds to a protonated form of TIM. For this reason the observed inhibition constant was adjusted to only account for the concentration of inhibitor trianion. By studying the pH dependence of the tri-ionic inhibitor binding constant it is possible to determine the pK_a of the active E165 base in the enzyme-inhibitor complex. Since the inhibitor is a transition state analogue, this pK_a value sheds light on the perturbation of the pK_a E165 upon substrate binding and loop 6 closure.

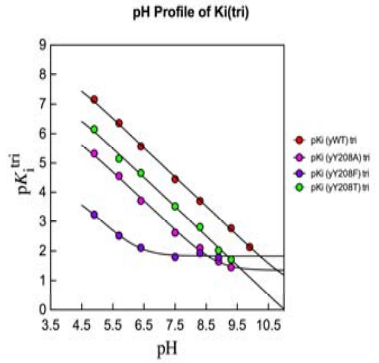
Y208A, Y208F and Y208T mutants were studied through PGA inhibition assays to understand the role of Y208 in the stabilization of the closed, active conformation and its pK_a perturbation of the E165.



Tyrosine (Y), aniline (A), phenylalanine (F) and tyrosine (T) structures (left to right).

$$K_i = \frac{(K_i)_E\left(1+\frac{[H^+]}{K_{EH}}\right)}{\left(1+\frac{[H^+]}{K_{EHI}}\right)} \qquad pK_i = \frac{10^{-p(K_i)_E}(1+10^{\frac{pH}{pK_{EH}}})}{1+10^{\frac{pH}{pK_{EHI}}}}$$

Equation 1 and 2: Inhibitor binding constant (K_i) and pK_i pH dependence.



Plot of pKi_{tri} versus the pH. The yY208F mutant shows the breakage at around pH 7.5. The equation is fitted to equation 2.

DISCUSSION AND CONCLUSION

- The pK_a of the carboxylic acid side chain is obtained from the position of the break in the pH profile. This pK_a is perturbed from 9.9 to 7.5 for the Y208F mutant and to a value of 9.5 for the Y208A mutant.
- The change in pK_a of E165 indicates that the Y208 residue is essential for the stabilization of the close active conformation of TIM through hydrogen bonding with loop7.
- The Y208A has a smaller effect on the activity of TIM than Y208F, suggesting that the effect of the Y208F mutation is due to more than a deletion of a single H-bond.

WORKS CITED

(1)Alber, T.; Banner, D. W.; Bloomer, A. C.; Petsko, G. A.; Phillips, D.; Rivers, P. S.; Wilson, I. A. On the Three-Dimensional Structure and Catalytic Mechanism of Triose Phosphate Isomerase. *Phil. Trans. R. Soc. Lond. B.* 1981, *293*, 159-171.

(2) Cui, Q.; Karplus, M. Triosephosphate: A Theoretical Comparison of Alternate Pathways. *J. Am. Chem. Soc.* 2001, *123*, 2284-2290.

(3) Malabanan, M. M.; Nitsch-Velasquez, L.; Amyes, T. L.; Richard, J. P.; Magnitude and Origin of the Enhanced Basicity of the Catalytic Glutamate of Triosephosphate Isomerase. *J. A. Chem.* 2013, *134*, 5978-5981.

(4) Amyes T. L.; O' Donoghue A. C.; Richard, J.P. Contribution of Phosphate Intrinsic Binding Energy to the Enzymatic Rate Acceleration forTriosephosphate Isomerase. *J. Am. Chem. Soc.* 2001, *123*, 11325-11326.

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