Sterics of conserved residue K422 in *Thermotoga maritima* phosphoglucose isomerase affect catalytic activity

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Abstract

Phosphoglucose isomerases (PGIs) are a class of enzymes that catalyze the reversible isomerization of glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P) via an acid-base catalyzed mechanism. This project was a structure-function study of the PGI from *Thermotoga maritima* where a strictly conserved basic residue, lysine (pKa = 10.79) was mutated to another basic amino acid, histidine (pKa = 6.04) at site 422. Previous studies suggested K422 plays a role in substrate binding. It was hypothesized the K422H point mutation would improve the catalytic efficiency through an increase in binding affinity. Kinetic assays did not align with the hypothesis as the catalytic efficiency for K422H decreased 6-fold compared to the wild-type. Binding affinity was moderately improved, however k_{cat} decreased significantly, indicating K422 likely serves a catalytic role. Other concurrent studies indicated the sterics of residue 422 affect the rate of catalysis. This understanding of K422 can be extrapolated to the understanding of other structurally conserved PGIs.

K422 Conserved at Active Site

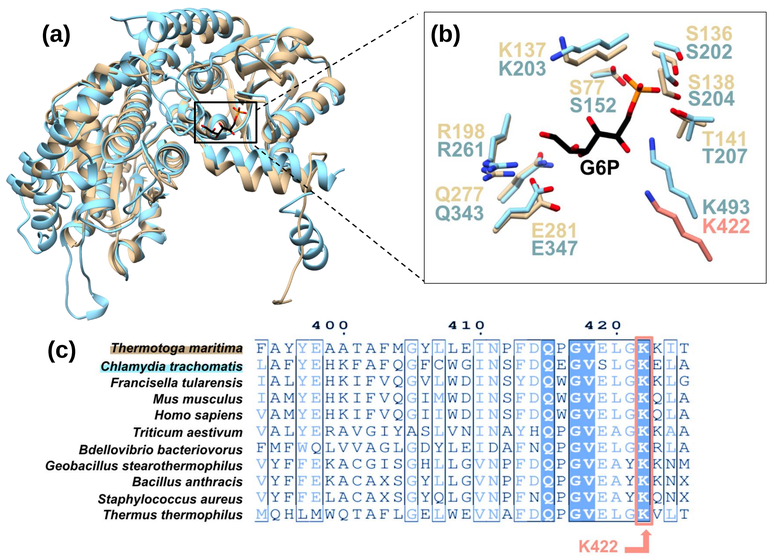


Figure 1. Conservation of K422 is demonstrated through (a) 3D structure alignment between *Thermotoga maritima* phosphoglucose isomerase (TmaPGI (PDB: 2Q8N)) and *Chlamydia trachomatis* phosphoglucose isomerase (CtrPGI (PDB: 6OTU)) crystalized with glucose-6-phosphate (G6P) substrate shows tertiary structure overlap. The RMSD for the aligned TmaPGI and CtrPGI was 1.001 Å, which implies similarity. (b) Active site residues between TmaPGI and CtrPGI crystalised with G6P substrate have strong alignment using Chimera. (c) Espript generated partial multiple sequence alignment with TmaPGI and 10 homologous PGIs, including CtrPGI. The salmon box highlights the highly conserved K422.

Role of Size in Active Site

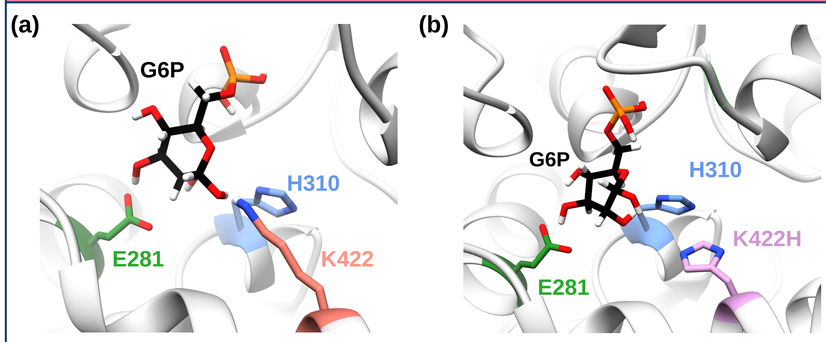


Figure 2. Active site of the wild-type and K422 mutant. (a) Features TmaPGI WT important residues K422, H310, and E281 with docked G6P. The orientation of G6P was chosen due to its orientation in the active site and a SP-dG of -6.8101. (b) Features the K422H mutation in TmaPGI with docked G6P, E281, and H310. This orientation of G6P was chosen due to its orientation in the active site and a SP-dG of -7.0151.

Methodology

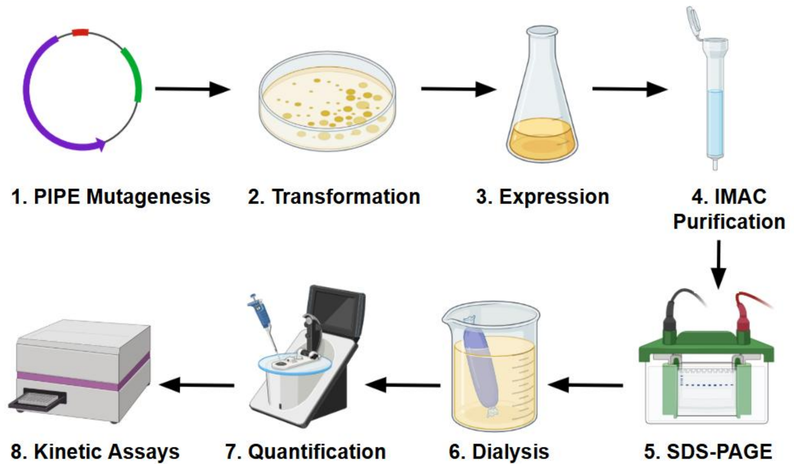


Figure 3. Schematic workflow for the preparation of WT and mutant protein. Recombinant protein was transformed with the mutagenized plasmid and expressed in HK100 *E. coli* cells. Protein expression was induced with arabinose. Purification was monitored by SDS-PAGE analysis. Protein was dialyzed and quantified prior to kinetic assays.

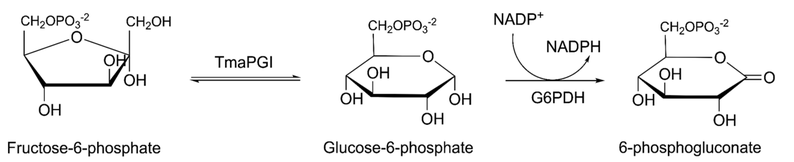


Figure 4. Reaction scheme for the coupled enzyme assay with glucose-6-phosphate dehydrogenase (G6PDH). Enzyme activity was analyzed by monitoring the change in [NADPH] via absorbance at 340 nm.

K422H Decreased Activity

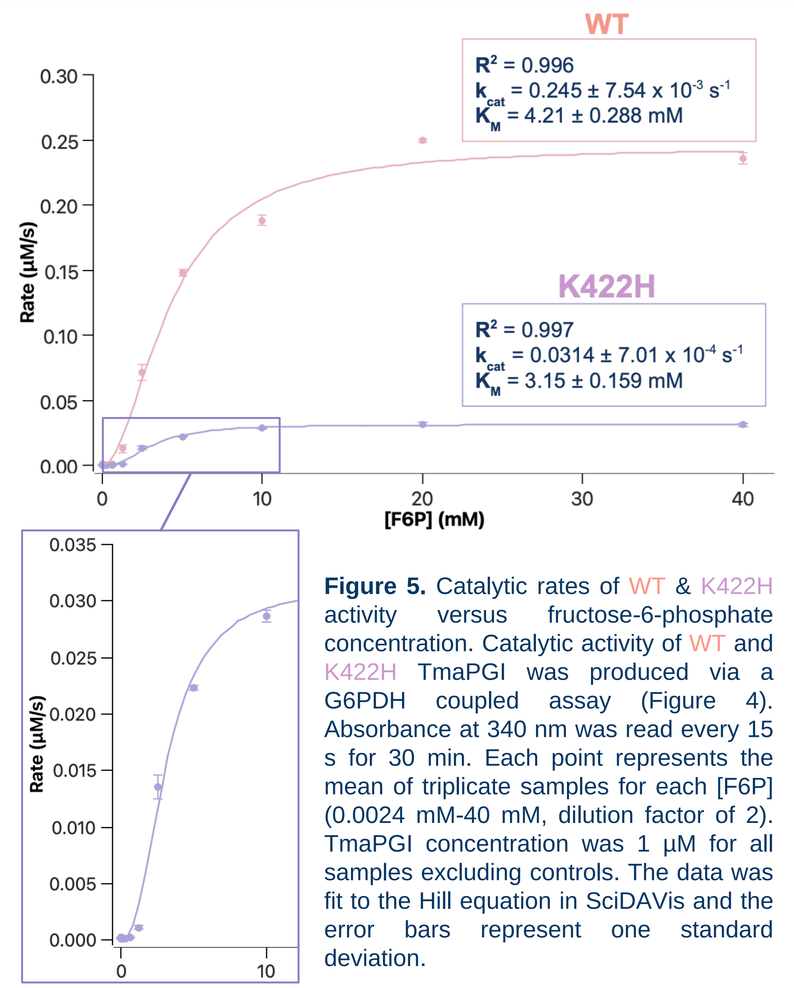
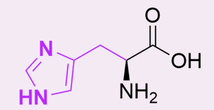
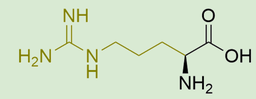
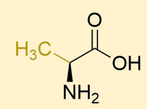


Figure 5. Catalytic rates of WT & K422H activity versus fructose-6-phosphate concentration. Catalytic activity of WT and K422H TmaPGI was produced via a G6PDH coupled assay (Figure 4). Absorbance at 340 nm was read every 15 s for 30 min. Each point represents the mean of triplicate samples for each [F6P] (0.0024 mM-40 mM, dilution factor of 2). TmaPGI concentration was 1 μM for all samples excluding controls. The data was fit to the Hill equation in SciDAVis and the error bars represent one standard deviation.

Kinetic Parameters Comparison

Table 1. Kinetic Parameters for F6P in G6PDH-Coupled Assay of WT and K422 Mutants.[a]

TmaPGI Variant		K_M	k_{cat}	Catalytic Efficiency[b]
WT		4.21 ± 0.29 mM	0.245 ± 0.008 s^{-1}	100%
K422H		3.15 ± 0.16 mM	0.0314 ± 0.0007 s^{-1}	17.1%
K422R		107%	12.0%	11.3%
K422A[c]		12.9%	5.64%	43.5%

[a]Kinetic data from K422R and K422A were obtained from separate studies conducted by other groups in the same lab; parameters for K422R and K422A were reported in relative change to their collected WT data. **Red** indicates unfavorable changes. **Green** indicates favorable changes.
[b]Catalytic efficiency is relative to wild-type and calculated as k_{cat}/K_M.
[c]Kinetic data was obtained from K422A mutation performed by group performing K422A/H310K double mutation.

Sterics May Impact Efficiency

- Position 422 is affected by the "bulk" of the residue present as demonstrated by the K_M, k_{cat}, and catalytic efficiency
  - Bulkier residues leads to lower efficiency (K422R<K422H<K422A)
- Demonstrated by the decreasing K_M values, lysine is more likely to play role in affinity than catalysis

Future Work

Table 2. Kinetic Parameters for K422H/H310A and K422A/H310A Mutations.

TmaPGI Variant	K_M	k_{cat}	Catalytic Efficiency[a]
K422H/H310A	48.5%	1.77%	3.66%
K422A/H310A[b]	NA	NA	NA

[a]Catalytic efficiency is relative to wild-type and calculated as k_{cat}/K_M. All percentages are reported in relative change with respect to wild-type data.
[b]K422A/H310A mutation data was obtained from Swope *et al.* NA indicates no activity.

Perform **K422H/H310K** double mutation to further explore interchanging roles of K422 and H310.

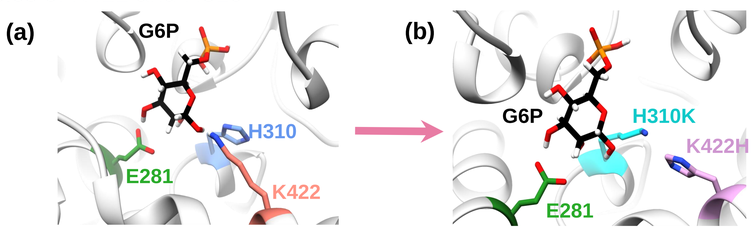


Figure 6. Active site of WT and K422H/H310K. (a) Features TmaPGI WT critical residues K422, H310, and E281 with docked G6P. The orientation of G6P was chosen due to its orientation in the active site and a SP-dG of -6.8101. (b) Features the K422H/H310K mutation in TmaPGI with docked G6P, H310, and E281.

References/Acknowledgements

Swope, N.; Lake, K. E.; Barrow, G. H.; Yu, D.; Fox, D. A.; Columbus, L. TM1385 from *Thermotoga Maritima* Functions as a Phosphoglucose Isomerase via Cis-Enediol-Based Mechanism with Active Site Redundancy. *BBA - Proteins and Proteomics* 2021, *1869* (4), 140602.
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