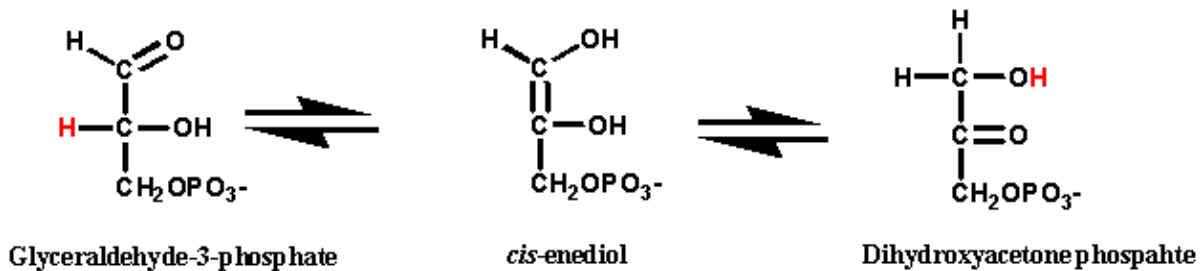


Chemical Mechanism of Triose Phosphate Isomerase

Triose phosphate isomerase represents the prototypical "TIM barrel" structure, an interior parallel α -barrel surrounded by β -helical interconnections between strands (/ barrel). The enzyme is a homodimer, each subunit having an active site near the carboxyl end of the barrel. As the enzyme name tells you, TIM (sometimes called "TPI" also) isomerizes triose phosphates; specifically, it interconverts glyceraldehyde 3-phosphate (an aldose) and dihydroxyacetone phosphate (the corresponding ketose) in glycolysis and gluconeogenesis. The isomerization can proceed in the absence of any catalyst, through an unstable enediol intermediate, but the uncatalyzed reaction is so slow that the aldose and the ketose are both stable compounds in the absence of a catalyst. A catalyst must increase the concentration of the enediol, by a) lowering the free energy of the enediol intermediate (stabilizing it and thus presumably also the transition state) and b) promoting the proton exchange required for enediol formation and thus increasing the rate.

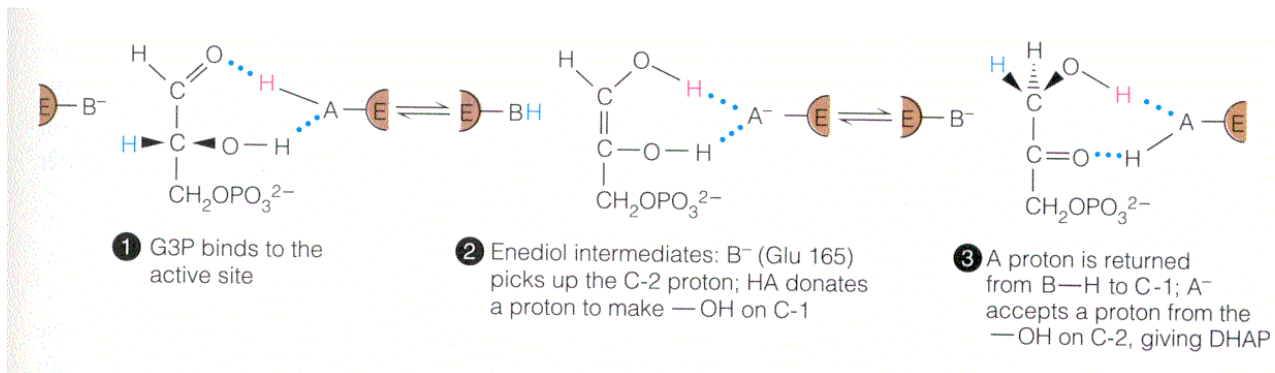
$G^{\circ} = -7.5$ kJ/mol for the reaction shown below, so the equilibrium favors DHAP. The value for k_{cat}/K_M in the glyceraldehyde 3-P \rightarrow DHAP direction is 4×10^8 M⁻¹•sec⁻¹. However, the reaction is reversible, and the active site accommodates either sugar as a substrate; the enzyme's chemical mechanism is reversible. *In vivo* the reaction is not at equilibrium; when glyceraldehyde 3-phosphate is being removed in glycolysis (glucose catabolism), DHAP is converted to glyceraldehyde 3-phosphate. When DHAP is needed for gluconeogenesis (glucose synthesis), glyceraldehyde 3-phosphate is converted to DHAP.



The triose phosphate isomerase mechanism illustrates

- Transition state stabilization
- Acid-base catalysis
- Electrostatic catalysis

The triose phosphate isomerase mechanism provides an example of **concerted acid-base catalysis**. The active site includes a Glu residue (Glu¹⁶⁵) thought to be the base (B^-) in the mechanism below, as well as a His residue (His⁹⁵, thought to be the HA in the mechanism below), and a Lys residue (Lys¹²). The enzyme provides a "cage" in which the substrate is held, by means of a "lid" (loop of the enzyme) that closes down upon the substrate to trap and protect the enediol intermediate from reacting with water. The catalytic groups are perfectly positioned to carry out acid-base catalysis and also to stabilize the enediol. Mutation of the Glu to an Asp, moving the catalytic COO^- group just 0.1 nm further from the substrate, results in a 1000-fold reduction in catalytic rate. The chemical mechanism is thought to proceed as follows:



The Glu- COO^- (B^-) extracts the proton from C-2 (so acts as a general base); simultaneously, the His imidazole group (AH) donates a proton to the C-1 carbonyl O (general acid), enhancing the rate of formation of the enediol. Then the Glu- $COOH$ donates its proton to C-1 (general acid), and the His accepts a proton from the C-2 OH group.