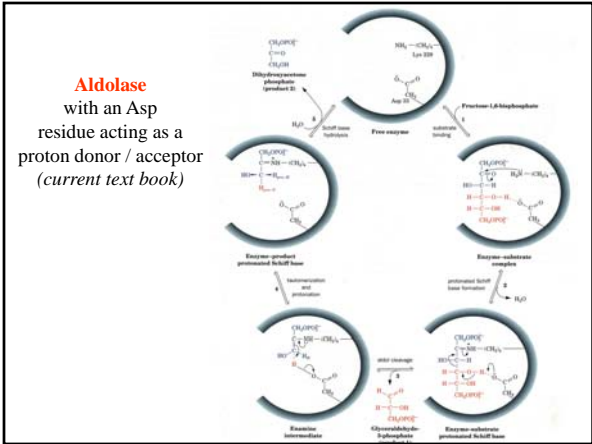
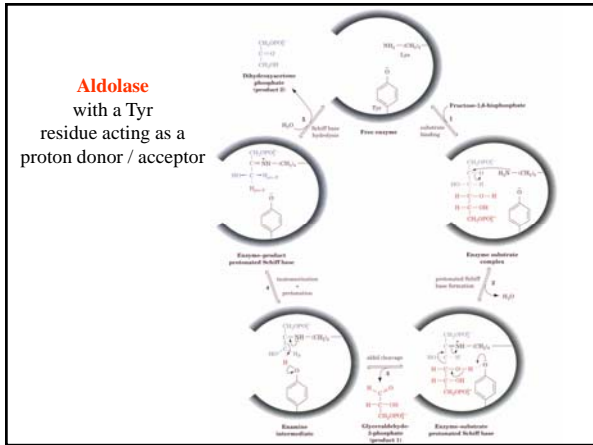
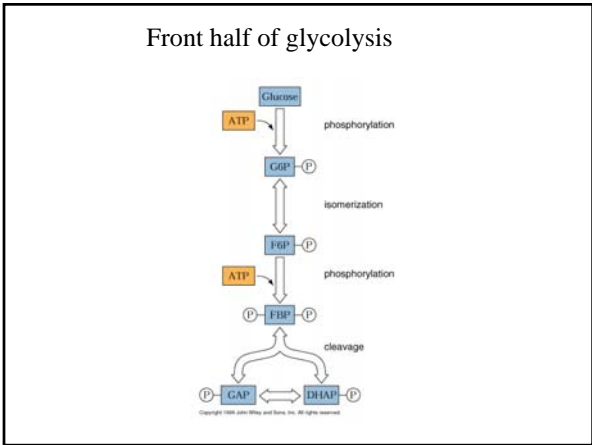
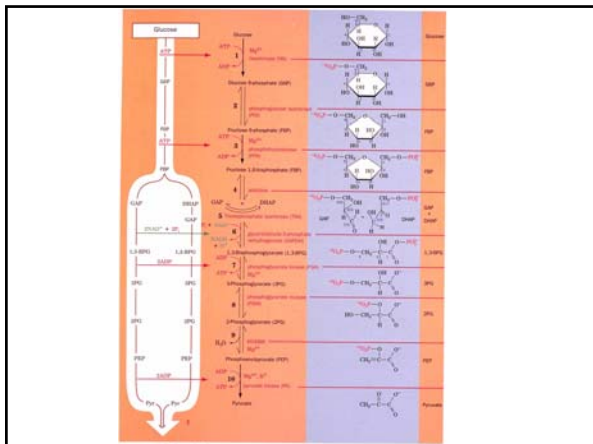


Glycolysis II
11/05/09



Triosephosphate isomerase

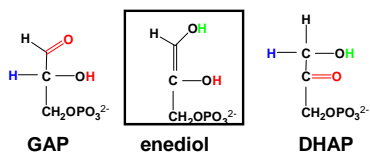
DHAP ↔ GAP

$$K_{eq} = \frac{[GAP]}{[DHAP]} = 4.7 \times 10^{-2} = \frac{1}{96}$$

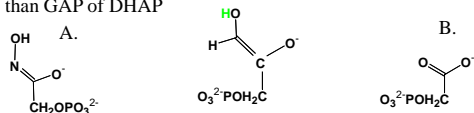
TIM is a perfect enzyme which its rate is diffusion controlled.

A rapid equilibrium allows GAP to be used and DHAP to replace the used GAP.

TIM has an enediol intermediate



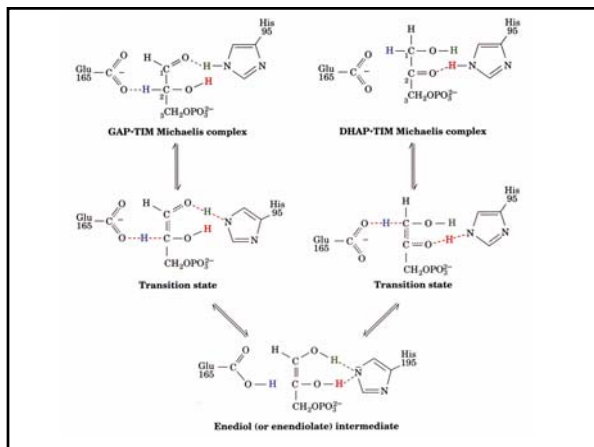
Transition state analogues Phosphoglycohydroxamate (A) and 2-phosphoglycolate (B) bind to TIM 155 and 100 times stronger than GAP of DHAP



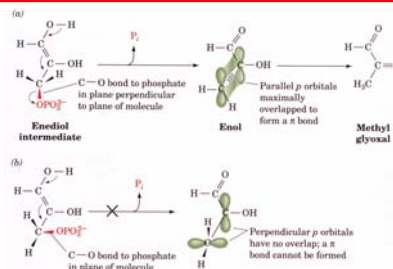
TIM has an extended "low barrier" hydrogen bond transition state



Hydrogen bonds have unusually strong interactions and have lead to pK of Glu 165 to shift from 4.1 to 6.5 and the pK of

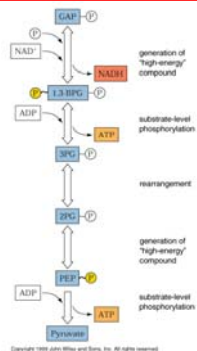


Geometry of the enediol intermediate prevents formation of methyl glyoxal



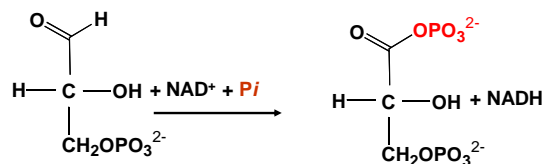
Orbital symmetry prevents double bond formation needed for methyl glyoxal

The second half of glycolysis



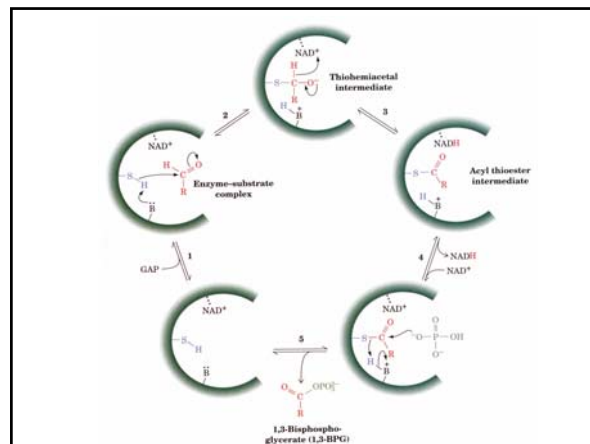
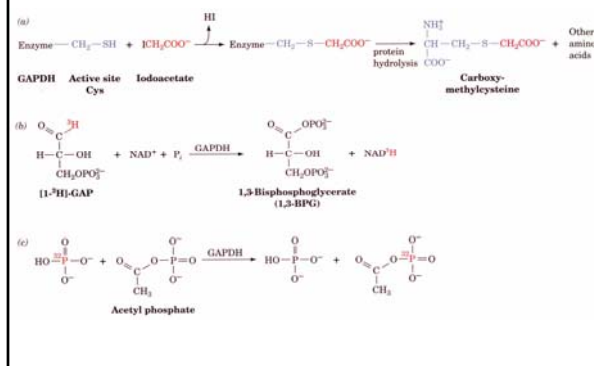
Glyceraldehyde-3-phosphate dehydrogenase

The first high-energy intermediate



Uses inorganic phosphate to create 1,3 bisphosphoglycerate

Reactions used to elucidate GAPDH's mechanism

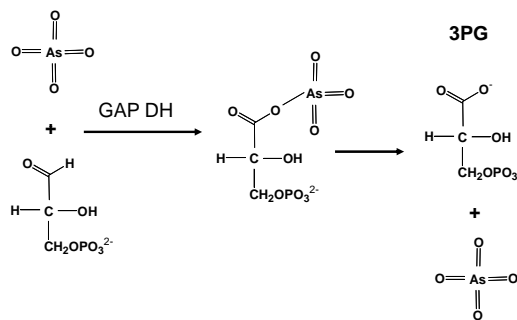


Mechanistic steps for GAPDH

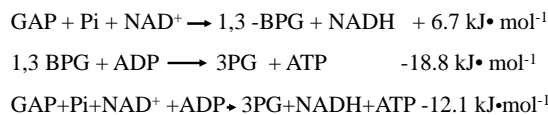
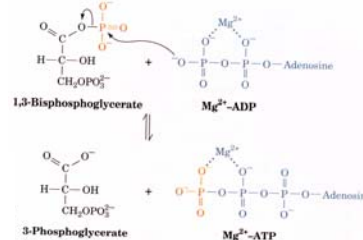
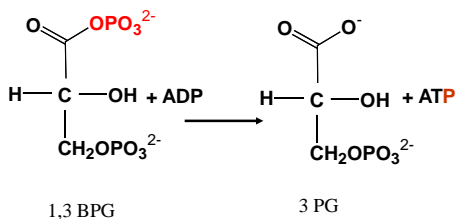
1. GAP binds to enzyme.
2. The nucleophile SH attacks aldehyde to make a thiohemiacetal.
3. Thiohemiacetal undergoes oxidation to an acyl thioester by a direct transfer of electrons to NAD⁺ to form NADH.
4. NADH comes off and NAD⁺ comes on.
5. Thioester undergoes nucleophilic attack by Pi to form 1,3 BPG.

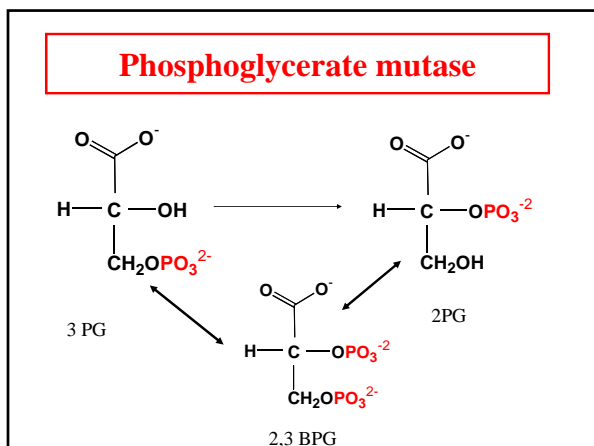
The acid anhydride of phosphate in a high energy phosphate intermediate

Arsenate uncouples phosphate formation



Phosphoglycerate Kinase: First ATP generation step

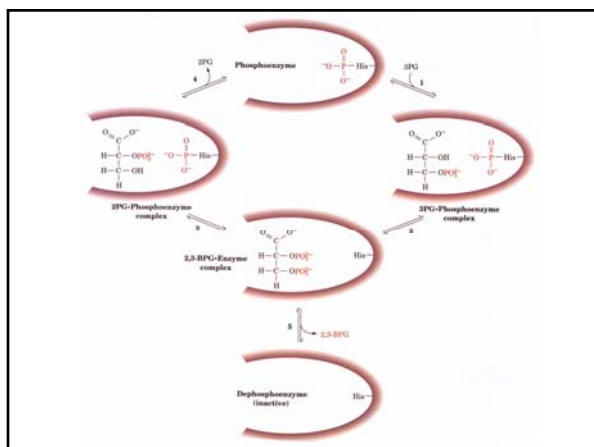




Phosphoglycerate mutase requires a phosphorylated form of the enzyme to be active. Only 2,3 BPG can phosphorylate the unphosphorylated enzyme.

Enzyme—H₂C——N—
PO₃²⁻

Phospho Histidine residue



Glycolysis influences oxygen transport

Glyceraldehyde 3-phosphate

1,3-Bisphosphoglycerate

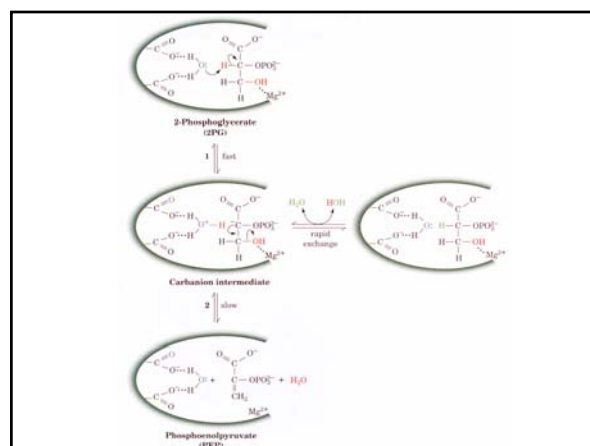
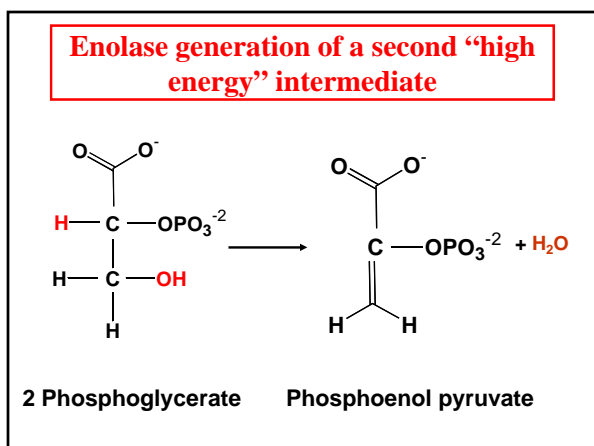
3-Phosphoglycerate

2-Phosphoglycerate

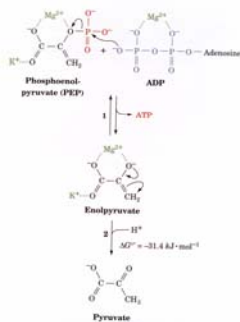
2,3-Bisphosphoglycerate (2,3-BPG)

3-Phosphoglycerate

Oxygen saturation curves in erythrocytes



Pyruvate kinase: Second ATP generation step



Next Lecture
Tuesday 11/10/09
Glycolysis III