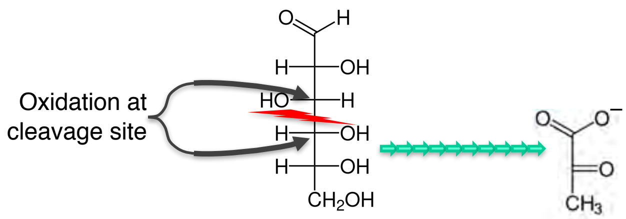
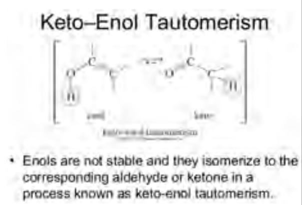
Glycolysis

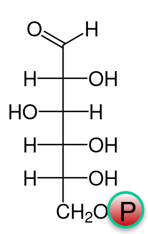




- It developed before photosynthesis, when the atmosphere was still anaerobic.
- Thus, the task upon early organisms was how to extract free energy from glucose anaerobically.
- Some of the free energy is captured in the synthesis of ATP and NADH.
- The solution:
  - First: All intermediates are phosphorylated: keeps them in the cell and "Activates" them for chemical degradation.
  - Second: Need to split in middle to make pathway convergent
  - Third: Overall oxidation (loss of only 4 e^-)
  - Fourth: Keto-enol tautomerization is key
  - Fifth: Collect energy from the high-energy metabolites.

Glycolysis: Hexokinase

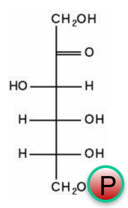
First Phosphorylation



- Rationale
  - traps glucose inside the cell
  - lowers intracellular (unphosphorylated) glucose concentration to allow further uptake by facilitative diffusion through GLUTS.
- This process uses the energy of ATP.
- Multiple isoforms of hexokinase exist in organisms (e.g., hexokinase I, II, III, and IV (glucokinase)).
- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphate of ATP.
- ATP-bound Mg^{++} facilitates this process by shielding the negative charges on ATP.
- Highly thermodynamically favorable/irreversible
  - $\Delta G^{o\prime} = -4$ kcal/mol
  - regulated mainly by product inhibition

Glycolysis: Phosphoglucose isomerase (PGI)

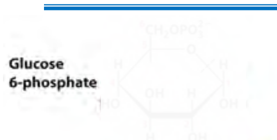
Setting up Second Phosphorylation

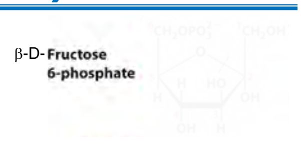


- Rationale
  - Convert C1 from aldehyde to hydroxyl which is easier to phosphorylate
- Slightly thermodynamically unfavorable/reversible
  - $\Delta G^{o'}$ = +0.4 kcal/mol
  - product concentration kept low by pairing with favorable next step to drive reaction forward

Glycolysis: Phosphoglucose Isomerase (PGI)

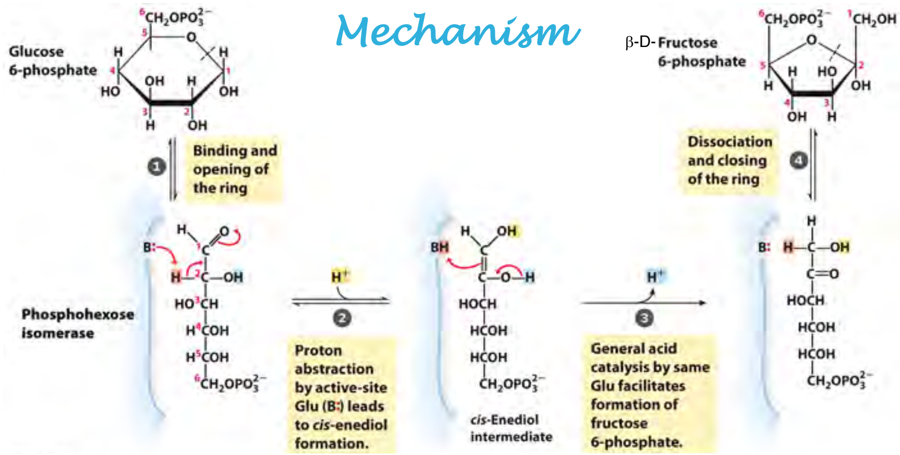
Mechanism





- The isomerization is catalyzed by the active-site glutamate via general acid/base catalysis.
- An aldose (glucose) can isomerize into a ketose (fructose) via an enediol intermediate.

Glycolysis: Phosphoglucose isomerase (PGI)



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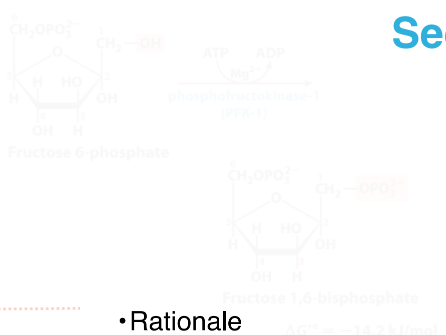
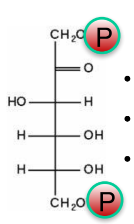
Glycolysis: Phosphofructokinase-1

Second Phosphorylation

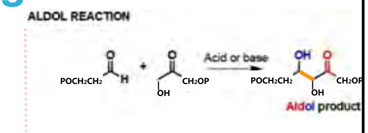


- Rationale
  - Symmetrical phosphorylation to set up cleavage to 3-carbon sugar
- First committed step of glycolysis
  - fructose 1,6-bisphosphate (Fru 1,6-P_2) is committed to become pyruvate and yield energy
- This process uses the energy of ATP.
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime}$ = –3.4 kcal/mol)
- Phosphofructokinase-1 is highly regulated.
  - by ATP, fructose 2,6-bisphosphate, and other metabolites
  - do not burn glucose if there is plenty of ATP

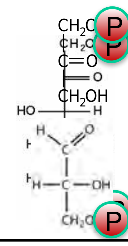


Glycolysis: Fructose-1,6-bisphosphate aldolase

Aldol Cleavage/condensation

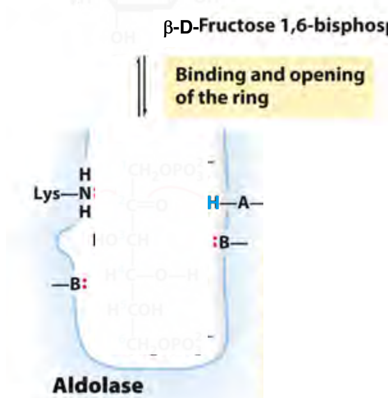


- Rationale
  - Six-carbon sugar is cleaved into two three-carbon phosphorylated sugars.
- The reverse process is the familiar aldol condensation.
- Multiple isoforms of aldolase exist; aldolase A, B, C.
- Multiple mechanisms to result in same product (i.e., convergent evolution):
  - Animal and plant aldolases employ covalent catalysis.
  - Fungal and bacterial aldolases employ metal ion catalysis.
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +5.7 kcal/mol)
  - product (GAP) concentration kept low to pull reaction forward.



Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism Covalent Catalysis (Class I enzymes)

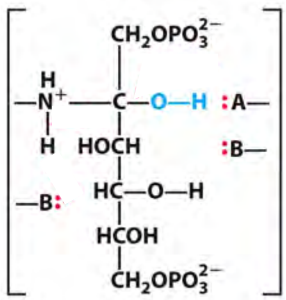


Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism

Loss of water produces Schiff base

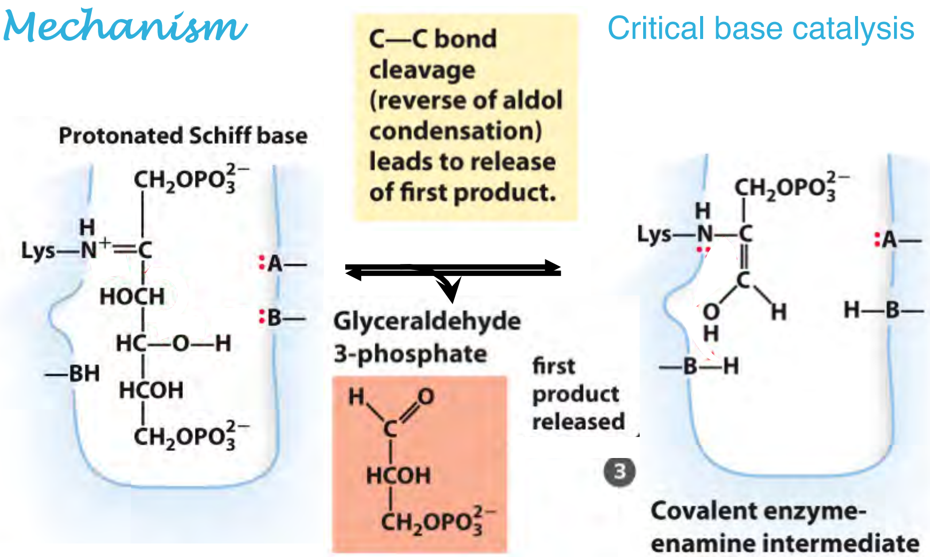
Protonated Schiff base



Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism

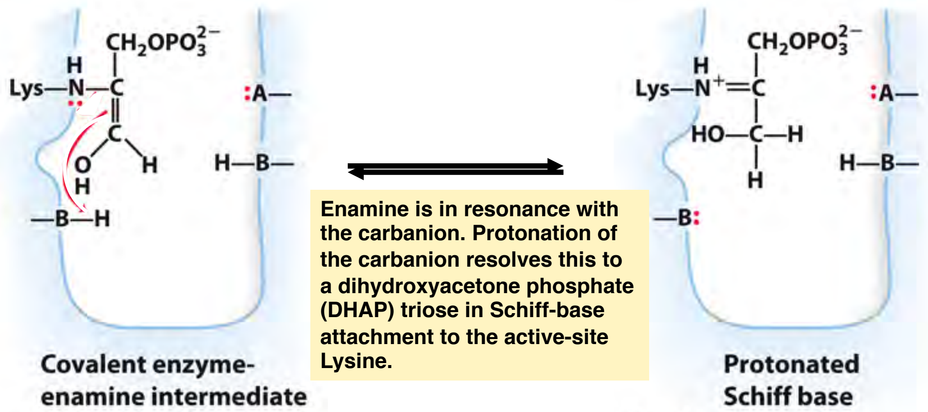
Critical base catalysis



Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism

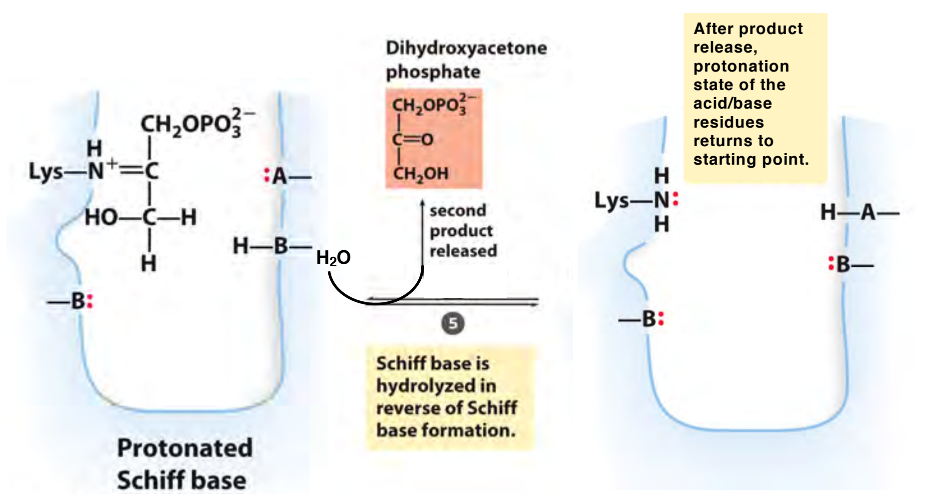
Acid catalysis protonates the carbanion



Glycolysis: Fructose-1,6-bisphosphate aldolase

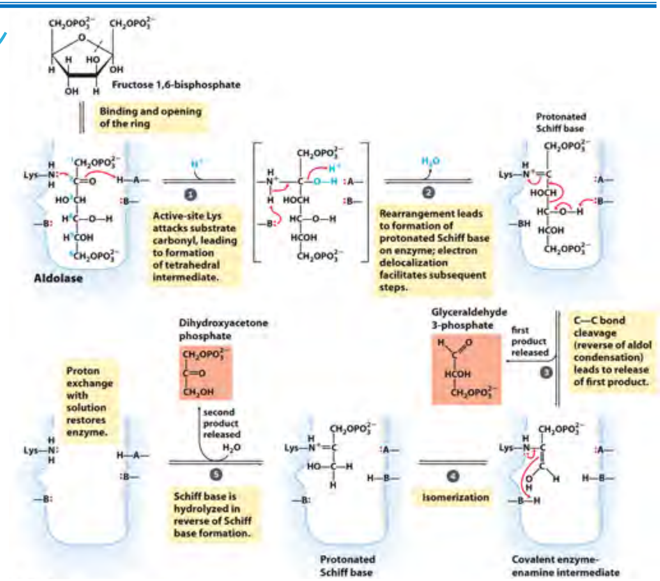
Mechanism

Product Release is the slow step



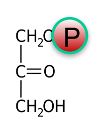
Glycolysis:
Fructose-1,6-bisphosphate aldolase

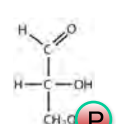
Mechanism Summary



Glycolysis:
Triose-phosphate isomerase (TIM)

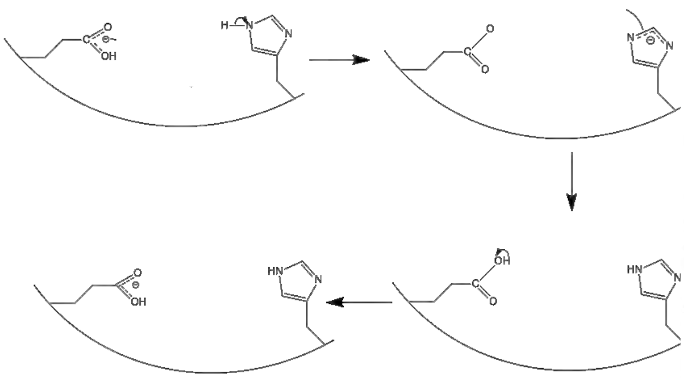
- Aldolase creates two triose phosphates:
  - glyceraldehyde-3-phosphate (GAP) – an aldose
  - dihydroxyacetone phosphate (DHAP) – a ketose
- Rationale:
  - allows convergence to a single chemical pathway (the payoff)
  - Need to oxidize C1 (formerly C3 of glucose) to carboxylate
- Completes preparatory phase of glycolysis
- Uses same enediol intermediate mechanism as PGI
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - GAP concentration kept low to pull reaction forward





Glycolysis: Triose-phosphate isomerase

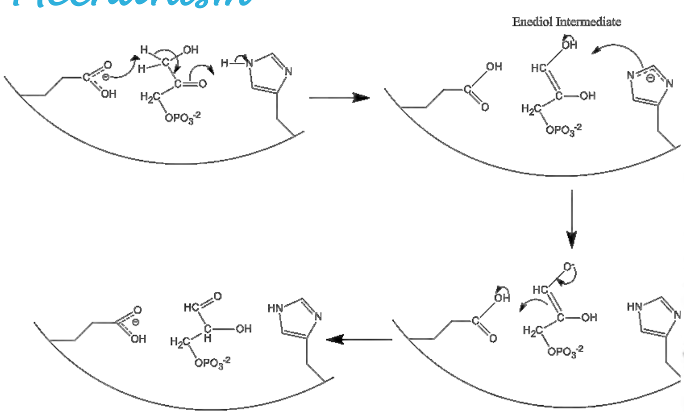
Mechanism

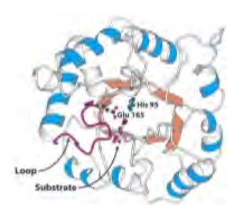


TIM caps enediol with loop around P_i so that elimination side reaction to form methyl glyoxal is not a good leaving group.

Glycolysis: Triose-phosphate isomerase

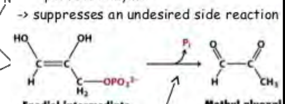
Mechanism





Triose phosphate isomerase (TPI)

- -> Isomerisation accelerated 10^{10}-fold
- -> $K_{cat}/K_m = 2*10^8\ M^{-1}\ s^{-1}$ -> kinetically perfect enzyme
- -> suppresses an undesired side reaction

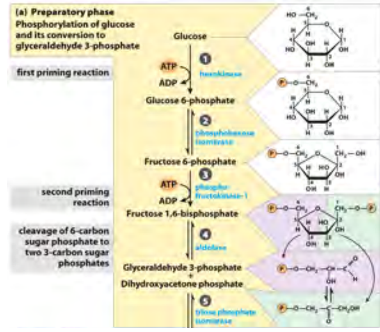


Reaction 100 times faster

TIM caps enediol with loop around P_i so that elimination side reaction to form methyl glyoxal is not a good leaving group.

Glycolysis: Overview

- Two Phases/Four concepts
  - Preparatory phase
    - Phosphorylation by ATP
    - Cleavage
  - Payoff
    - Oxidation
    - Phosphorylation of ATP



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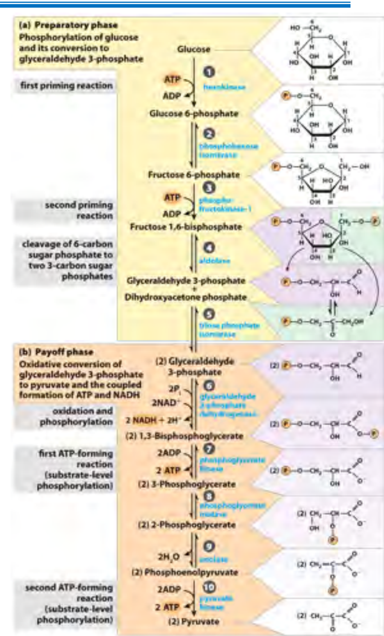
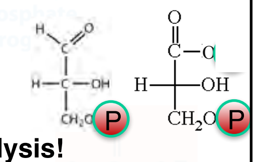


Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



- Rationale:
  - Recall Pyruvate is an acid; need to oxidize aldehyde
  - incorporates inorganic phosphate
  - generation of a high-energy phosphate compound
  - **which allows for net production of ATP via glycolysis!**
- First energy-yielding step in glycolysis
- First oxidation: aldehyde to carboxylate (ox)/NAD$^+$ to NADH (red).
- Active-site cysteine
  - forms high-energy thioester intermediate
  - subject to inactivation by oxidative stress
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - coupled to next reaction to pull forward

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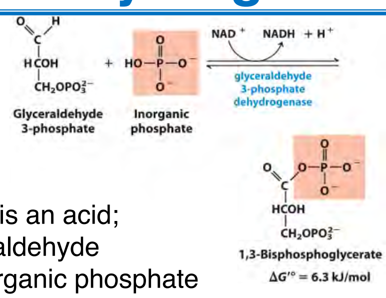

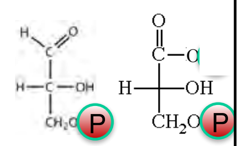

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