BB 422/622

OUTLINE:

- Review of 421
- Goals of 422
- Review of chemical principles
  - Thermodynamics
  - C/O cycles
  - Overview of Metabolism
  - ATP cycles
- Energy Coupling
- Chemical Reactivity
- Bioenergetics
- Membrane Transport
  - Review of membrane structure, dynamics, and proteins
  - Mediated/non-mediated
  - Energetics
  - Facilitative Diffusion
    - ionophores
    - GLUT1
    - Aquaporins
    - Potassium channel
  - Active Transport
    - Primary
      - Na/K pump
      - ABC transporters
    - Secondary
      - Glc import
      - Bicarbonate/Cl
      - Lactose/H+
- Catabolism of Glucose
- Glycogenolysis
  - phosphorylase
  - debranching enzyme
  - phospho-gluco-mutase (PGM)
- Glycolysis
  - Introduction & overview;
  - Phase I
    - hexokinase
    - phospho-gluco-isomerase (PGI)
    - phospho-fructo-kinase (PFK-1)
    - Aldolase
    - triose-phosphate isomerase (TPI)

Exam 1

  - Phase II

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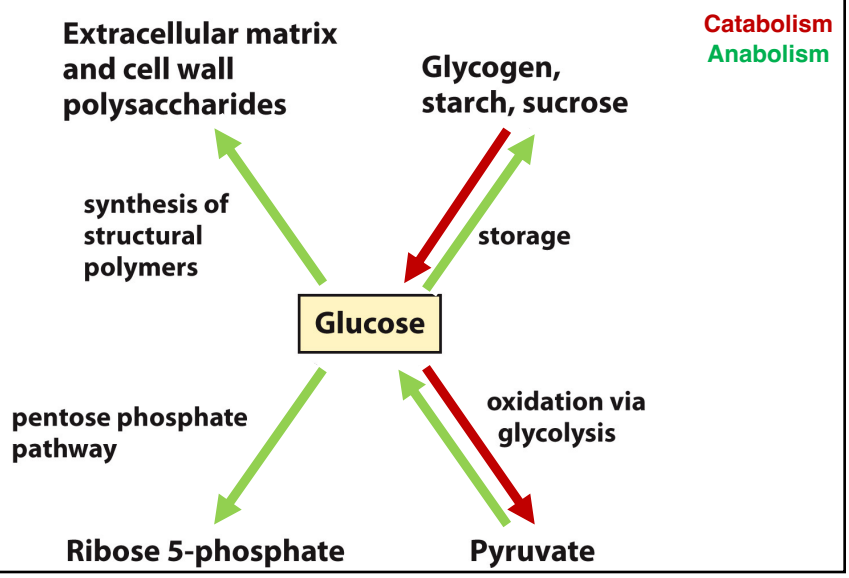
Glucose Utilization

Four Major Fates of Glucose



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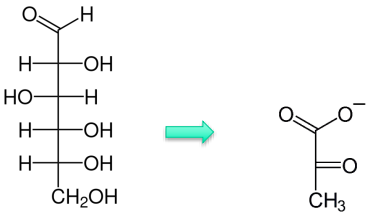
Glycolysis



- Sequence of enzyme-catalyzed reactions by which glucose is converted into pyruvate
  - Pyruvate can be further aerobically oxidized.
  - Pyruvate can be used as a precursor in biosynthesis.
- In the evolution of life, glycolysis probably was one of the earliest energy-yielding pathways.
- Research of glycolysis played a large role in the development of modern biochemistry.
  - understanding the role of coenzymes
  - discovery of the pivotal role of ATP
  - development of methods for enzyme purification
  - inspiration for the next generations of biochemists

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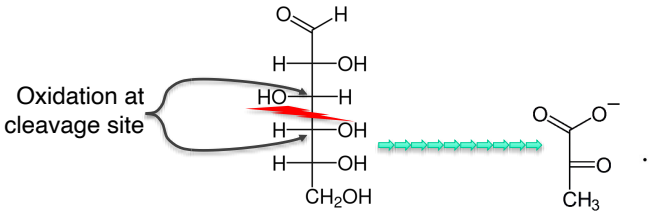
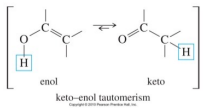
Glycolysis





- Enols are not stable and they isomerize to the corresponding aldehyde or ketone in a process known as keto-enol tautomerism.

- 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.

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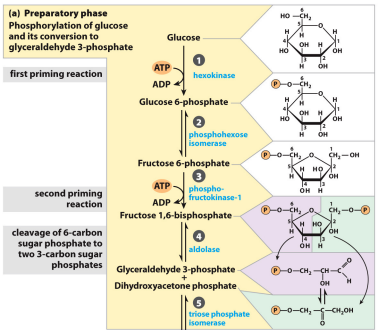
Glycolysis: Overview

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



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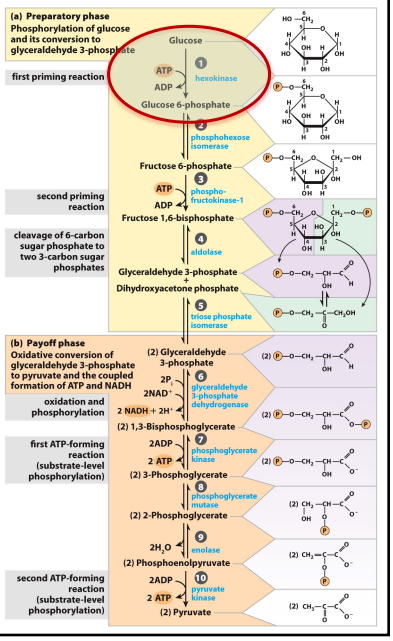
Glycolysis: Overview

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



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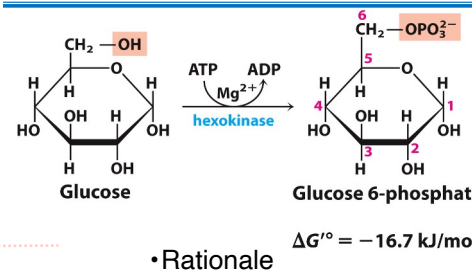
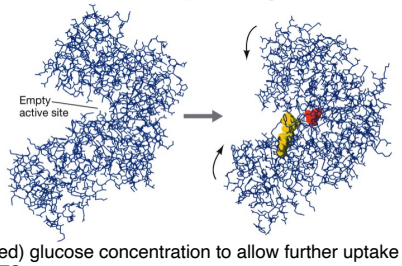
Glycolysis: Hexokinase



$\Delta G'^{\circ} = -16.7$ kJ/mol

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 (energy coupling).
- Multiple isoforms of hexokinase exist in organisms (e.g., hexokinase I, II, III, and IV (glucokinase; liver & pancreas)).
- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphate of ATP in S_N2 substitution (bi-pyramidal intermed.)
- ATP-bound Mg^{++} facilitates this process by shielding the negative charges on ATP.
- Highly thermodynamically favorable/irreversible
  - $\Delta G^{\circ\prime} = -4$ kcal/mol
  - regulated mainly by product inhibition

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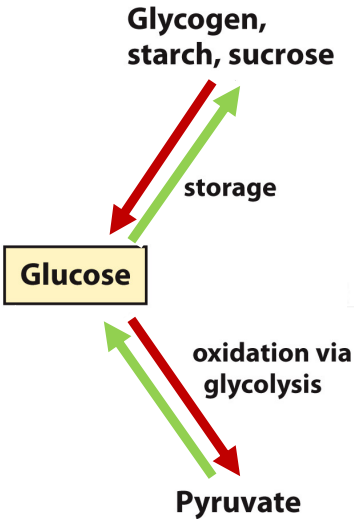
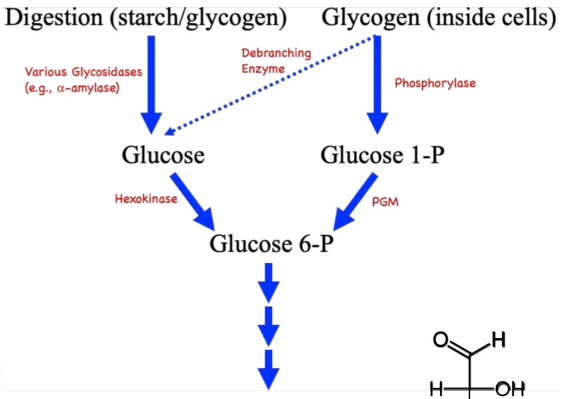
Glucose Utilization

Fates of Glucose

Catabolism

Anabolism





Glucose 6-phosphate is a key junction point

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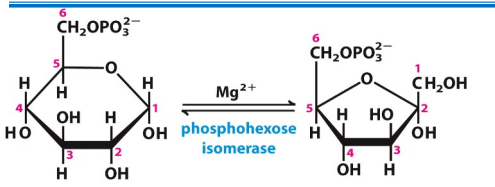
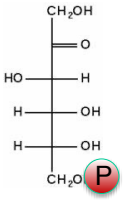
Glycolysis: Phosphoglucose isomerase (PGI)



Setting up Second Phosphorylation

$\Delta G'^{\circ} = 1.7$ kJ/mol



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

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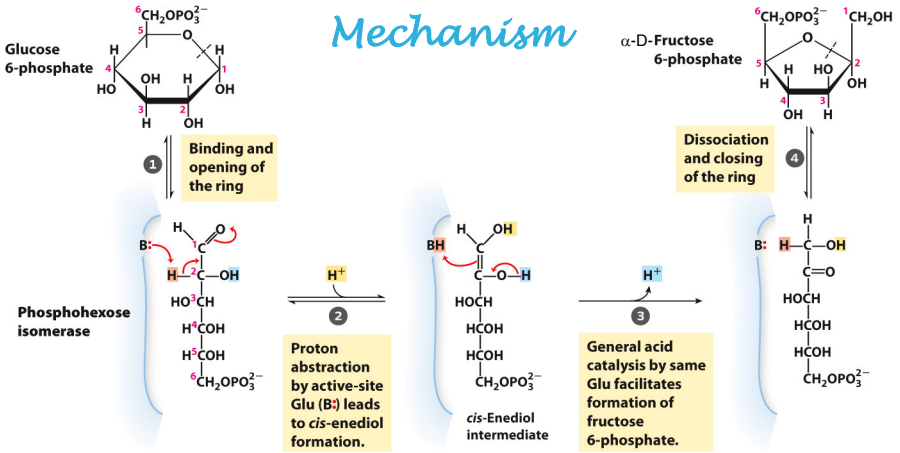
Glycolysis: Phosphoglucose isomerase (PGI)

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.

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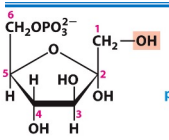
Glycolysis: Phosphofructokinase-1

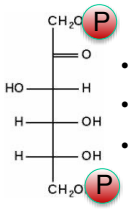


Fructose 6-phosphate

Fructose 1,6-bisphosphate

Second Phosphorylation

- Rationale $\Delta G'^{\circ} = -14.2$ kJ/mol
  - 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

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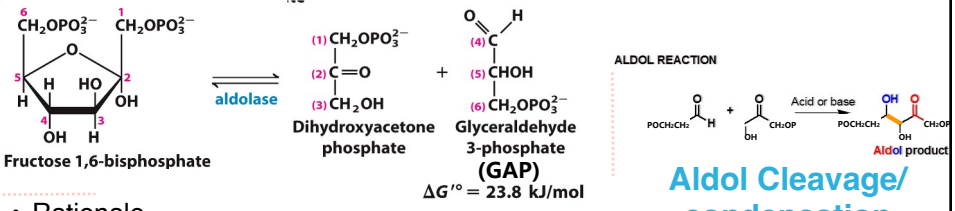
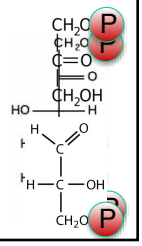
Glycolysis: Fructose-1,6-bisphosphate Aldolase



Fructose 1,6-bisphosphate

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 (Schiff base).
  - 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.



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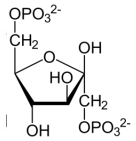
Glycolysis: Fructose-1,6-bisphosphate aldolase

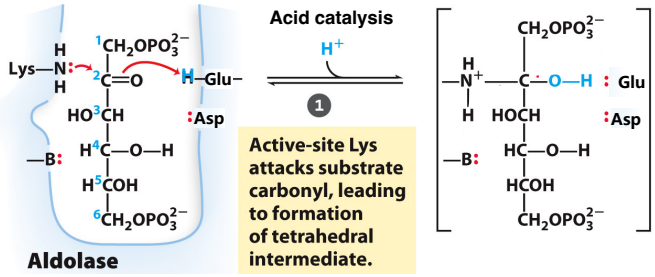
Mechanism Covalent Catalysis (Class I enzymes)

Step 1: making the Schiff Base



β-D-Fructose 1,6-bisphosphate

Binding and opening of the ring



Active-site Lys attacks substrate carbonyl, leading to formation of tetrahedral intermediate.

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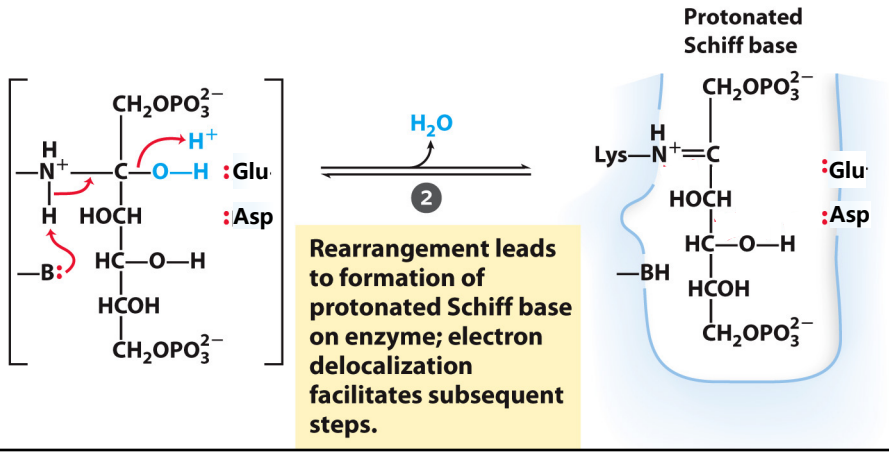
Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism Loss of water produces Schiff base

Step 1: making the Schiff Base



Rearrangement leads to formation of protonated Schiff base on enzyme; electron delocalization facilitates subsequent steps.

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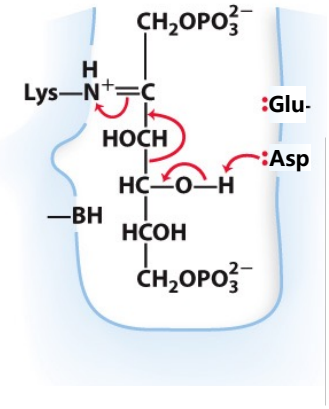
Glycolysis: Fructose-1,6-bisphosphate aldolase

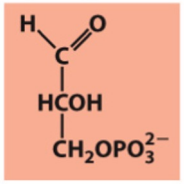
Mechanism
Step 2: C-C bond cleavage

Protonated Schiff base



C—C bond cleavage (reverse of aldol condensation) leads to release of first product.

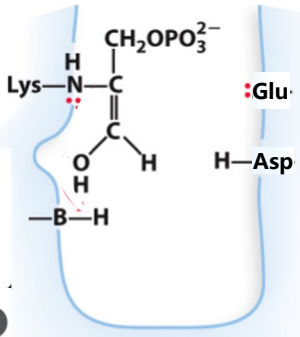
Glyceraldehyde 3-phosphate



first product released

3

Critical base catalysis



Covalent enzyme-enamine intermediate

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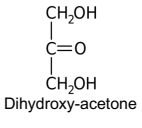
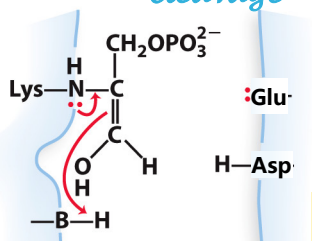
Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism
Step 2: C-C bond cleavage

Acid catalysis protonates the carbanion

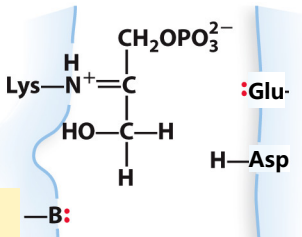


Dihydroxy-acetone



Covalent enzyme-enamine intermediate

Enamine is in resonance with the carbanion. Protonation of the carbanion resolves this to a dihydroxyacetone phosphate (DHAP) triose in Schiff-base attachment to the active-site Lysine.



Protonated Schiff base

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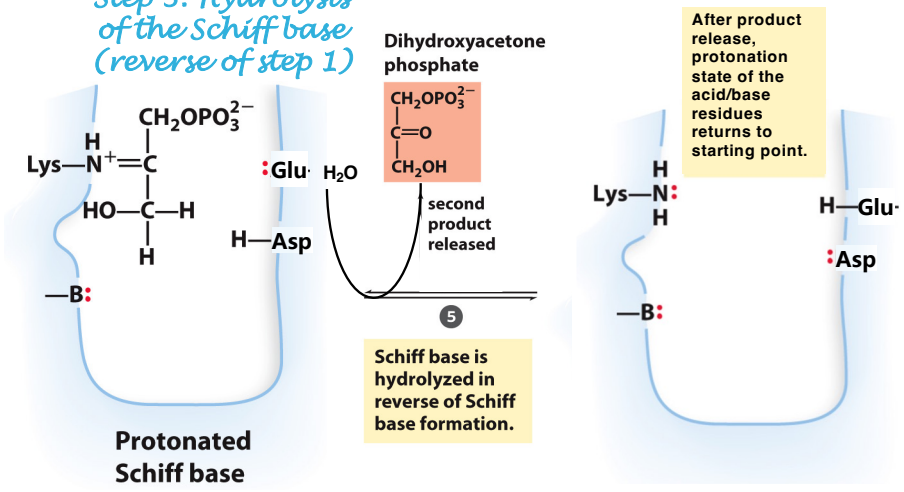
Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism

Step 3: Hydrolysis of the Schiff base (reverse of step 1)

Product Release is the slow step



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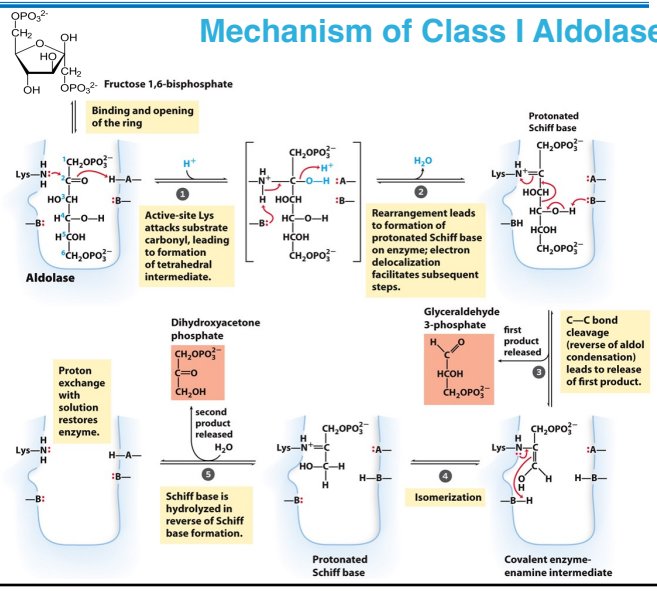
Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism Summary

Mechanism of Class I Aldolases



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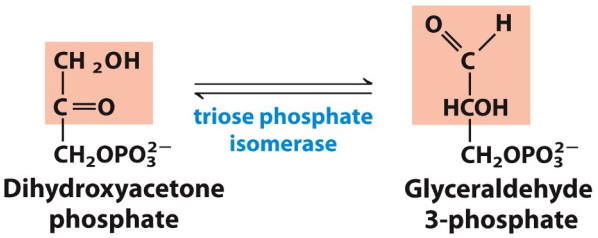
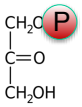
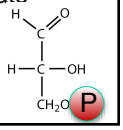
Glycolysis: Triose-phosphate isomerase (TIM)



Dihydroxyacetone phosphate ⇌ **Glyceraldehyde 3-phosphate**

- Aldolase creates two triose phosphates:
  - glyceraldehyde-3-phosphate (GAP) – an aldose
  - dihydroxyacetone phosphate (DHAP) – a ketose
- TIM completes preparatory phase of glycolysis
- Rationale:
  - allows convergence to a single chemical pathway (the payoff in phase II)
  - Need to oxidize C1 of GAP (formerly C3 & C4 of glucose) to carboxylate
- TIM uses same enediol intermediate mechanism as PGI
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +1.8 kcal/mol)
  - GAP concentration kept low to pull reaction forward





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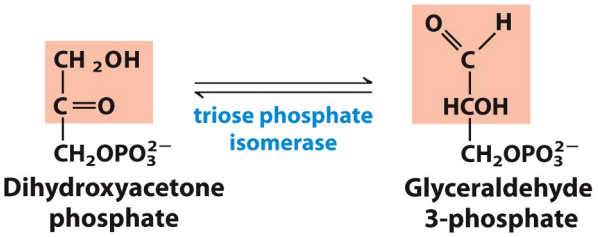
Glycolysis: Triose-phosphate isomerase (TIM)



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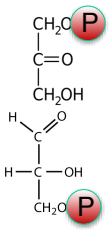

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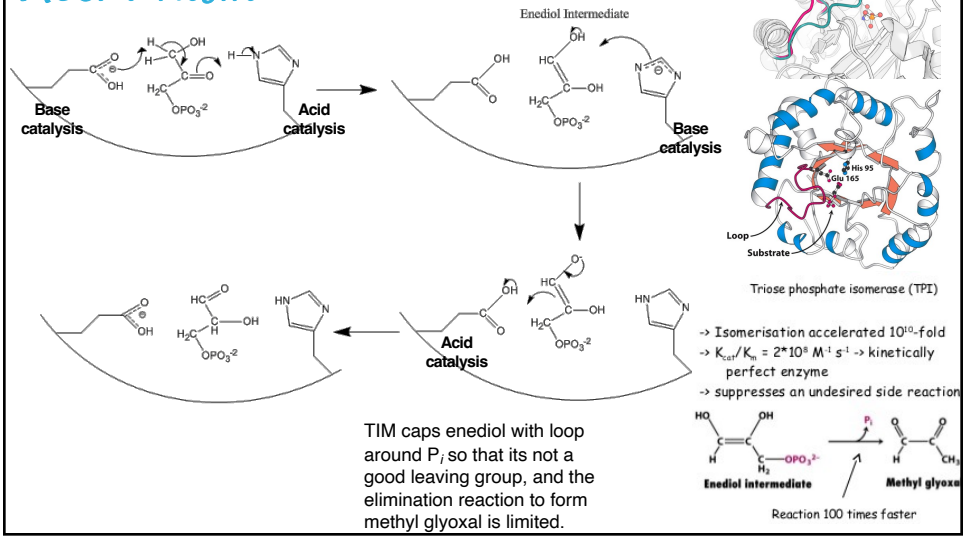
Glycolysis: Triose-phosphate isomerase

Mechanism



TIM caps enediol with loop around P_i so that its not a good leaving group, and the elimination reaction to form methyl glyoxal is limited.

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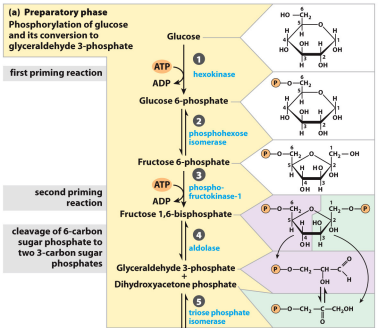
Glycolysis: Overview

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



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Material for Exam 1 Ends

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