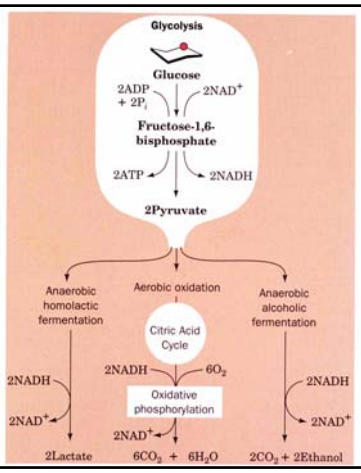
Glycolysis I
11/03/09

Glycolysis

The conversion of glucose to pyruvate to yield 2ATP molecules
- **10 enzymatic steps**
- **Chemical interconversion steps**
- **Mechanisms of enzyme conversion and intermediates**
- **Energetics of conversions**
- **Mechanisms controlling the Flux of metabolites through the pathway**



Historical perspective

Winemaking and baking industries

1854-1865 Louis Pasture established that microorganisms were responsible for fermentation.

1897 Eduard Buchner- cell free extracts carried out fermentation

no “vital force” and put fermentation in the province of chemistry

1905 - 1910 Arthur Harden and William Young

- inorganic phosphate was required ie. fructose-1,6-bisphosphate
- zymase and cozymase fractions can be separated by diaylsis

Inhibitors were used. Reagents are found that inhibit the production of pathway products, thereby causing the buildup of metabolites that can be identified as pathway intermediates.

Fluoride- leads to the buildup of 3-phosphoglycerate and 2-phosphoglycerate

1940 Gustav Embden, Otto Meyerhof, and Jacob Parnas put the pathway together.

Pathway overview

1. Add phosphoryl groups to activate glucose.

2. Convert the phosphorylated intermediates into high energy phosphate compounds.

3. Couple the transfer of the phosphate to ADP to form ATP.

Stage I A preparatory stage in which glucose is phosphorylated and cleaved to yield two molecules of glyceraldehyde-3-phosphate - uses two ATPs

Stage II glyceraldehyde-3-phosphate is converted to pyruvate with the concomitant generation of four ATPs-net profit is 2ATPs per glucose.

Glucose + 2NAD$^+$ + 2ADP +2P*i* → 2NADH + 2pyruvate + 2ATP + 2H$_2$O + 4H$^+$

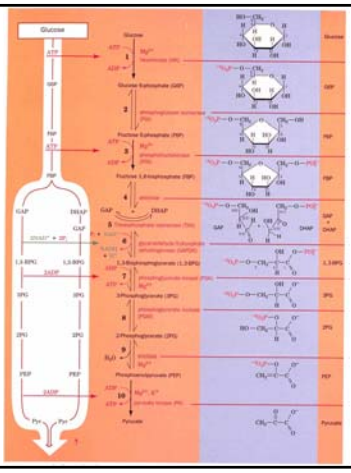
Oxidizing power of NAD+ must be recycled

NADH produced must be converted back to NAD+

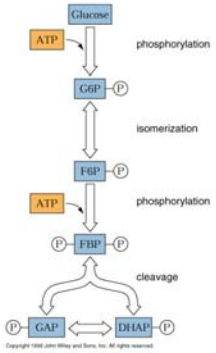
1. Under anaerobic conditions in muscle NADH reduces pyruvate to lactate (homolactic fermentation).

2. Under anaerobic conditions in yeast, pyruvate is decarboxylated to yield CO_2 and acetaldehyde and the latter is reduced by NADH to ethanol and NAD+ is regenerated (alcoholic fermentation).

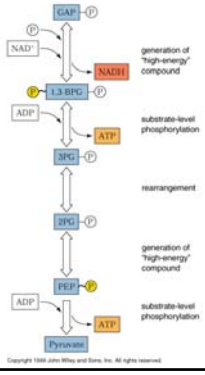
3. Under aerobic conditions, the mitochondrial oxidation of each NADH to NAD+ yields three ATPs



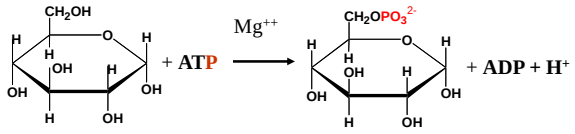
Front half of glycolysis



The second half of glycolysis



Hexokinase



Glucose **Glucose-6-phosphate**

Isozymes: Enzymes that catalyze the same reaction but are different in their kinetic behavior

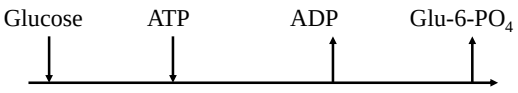
Tissue specific

Glucokinase- Liver controls blood glucose levels.

Hexokinase in muscle - allosteric inhibition by ATP

Hexokinase in brain - NO allosteric inhibition by ATP

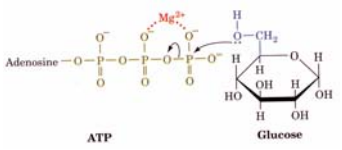
Hexokinase reaction mechanism is
RANDOM Bi-Bi

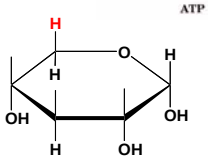


When ATP binds to hexokinase without glucose it does not hydrolyze ATP. WHY?

The binding of glucose elicits a structural change that puts the enzyme in the correct position for hydrolysis of ATP.

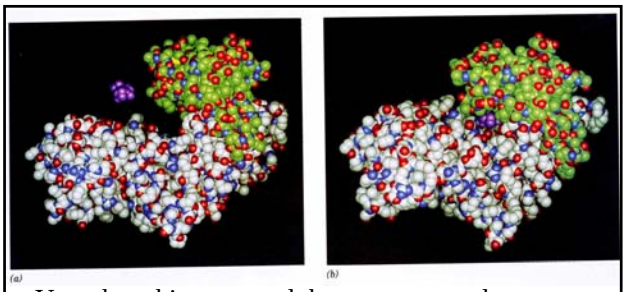
The enzyme movement places the ATP in close proximity to C_6H_2OH group of glucose and excludes water from the active site.





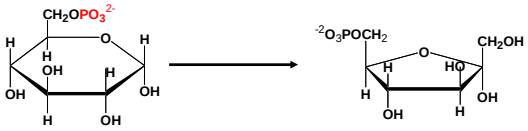
α-D-Xylose

There is a 40,000 fold increase in ATP hydrolysis upon binding xylose which cannot be phosphorylated!



Yeast hexokinase, two lobes are gray and green. Binding of glucose (purple) causes a large conformational change. A substrate induced conformational change that prevents the unwanted hydrolysis of ATP.

Phosphoglucose Isomerase



Uses an " ene dione intermediate

1) Substrate binding

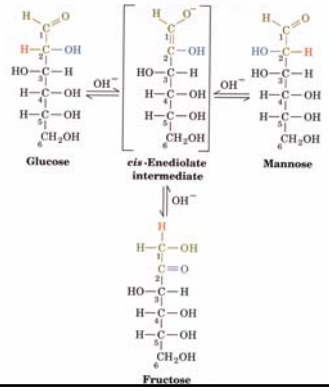
2) Acid attack by H_2N-Lys opens the ring

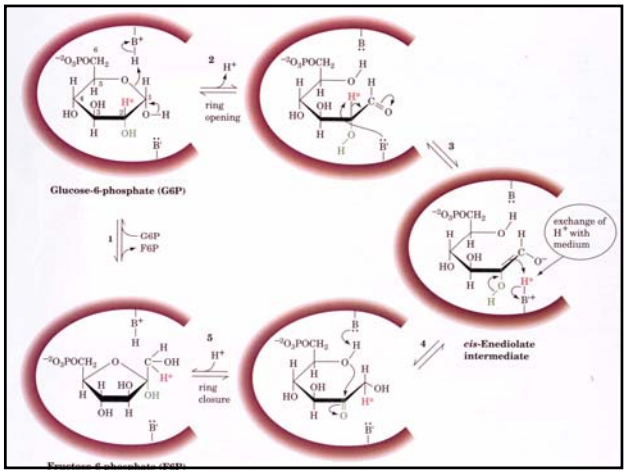
3) Base unprotonated Glu abstracts proton from C2

4) Proton exchange

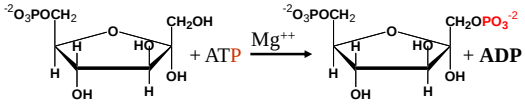
5) Ring closure

Uncatalyzed isomerization of Glucose





Phosphofructokinase



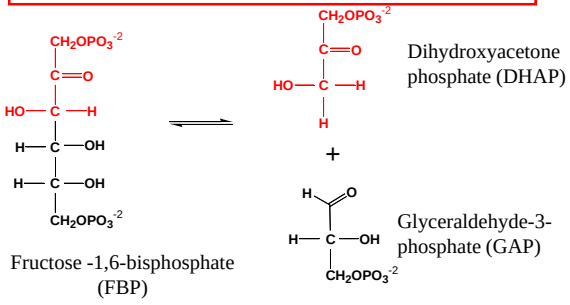
Fructose-6-PO_4 **Fructose-1,6-bisphosphate**

1.) Rate limiting step in glycolysis

2.) Irreversible step, can not go the other way

3.) The control point for glycolysis

Aldolase



Aldol cleavage (retro aldol condensation)

There are two classes of Aldolases

Class I animals and plants - Schiff base intermediate

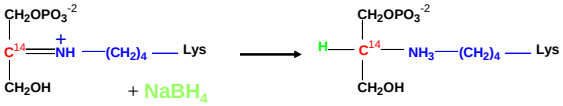
Step 1 Substrate binding

Step 2 FBP carbonyl groups reacts with amino LYS to form iminium cation (Schiff base)

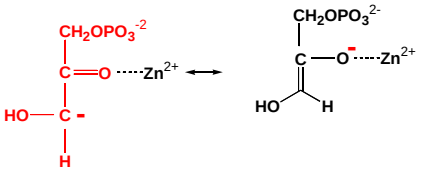
Step 3. C3-C4 bond cleavage resulting enamine and release of GAP

Step 4 protonation of the enamine to a iminium cation

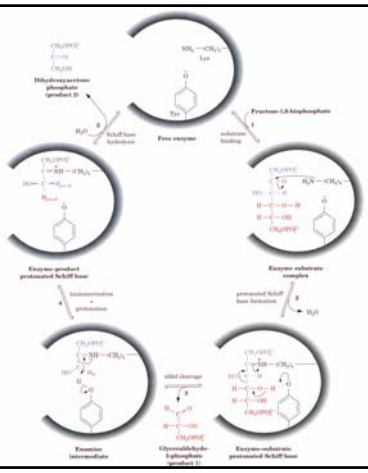
Step 5 Hydrolysis of iminium cation to release DHAP



Class II enzymes are found in fungi and algae and do not form a Schiff base. A divalent cation usually a Zn^{+2} polarizes the carbonyl intermediate.

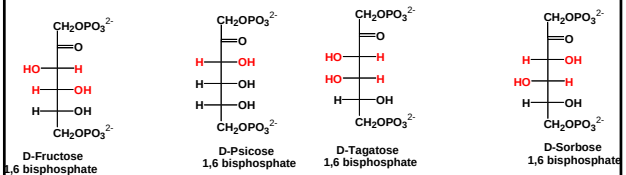


Probably the occurrence of two classes is a metabolic redundancy that many higher organisms replaced with the better mechanism.



Aldolase is very stereospecific

When condensing DHAP with GAP four possible products can form depending on the whether the *pro*-S or *pro* R hydrogen is removed on the C3 of DHAP and whether the *re* or *si* face of GAP is attacked.



Triosephosphate isomerase

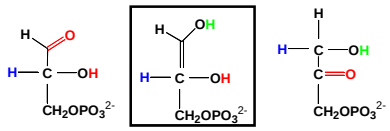
DHAP ⟷ GAP

$$K_{eq} = \frac{[GAP]}{[DHAP]} = 4.7x10^{-2} = \frac{1}{96}$$

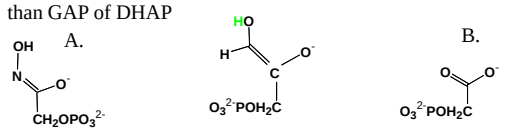
TIM is a perfect enzyme which its rate is diffusion controlled.

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

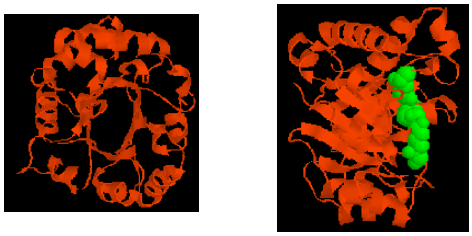
TIM has an enediol intermediate



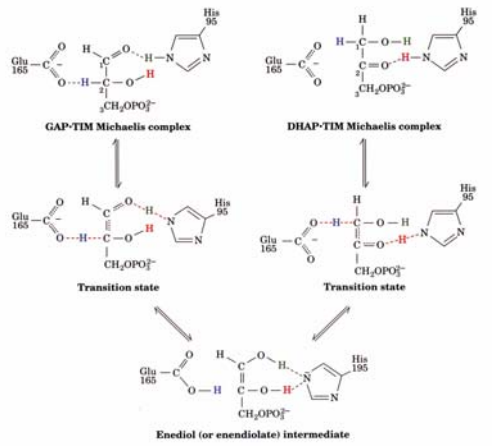
Transition state analogues Phosphoglycohydroxamate (A) and 2-phosphoglycolate (B) bind to TIM 155 and 100 times stronger than GAP of DHAP



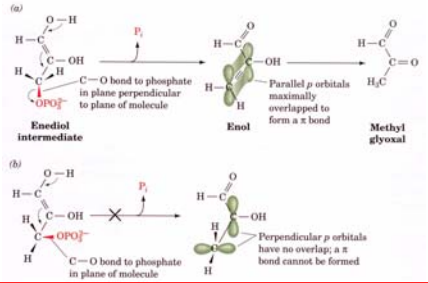
TIM has an extended "low barrier" hydrogen bond transition state



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

Next Lecture
Thursday 11/05/09
Glycolysis II