Glycolysis Review

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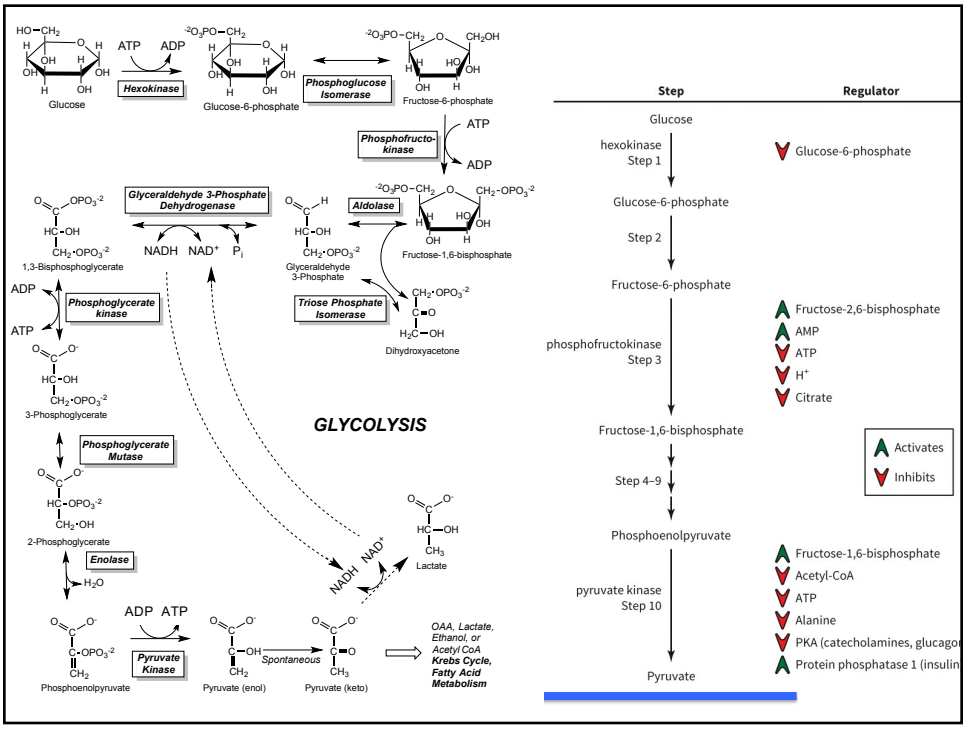

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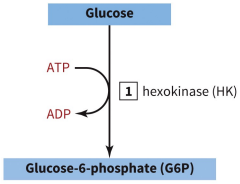
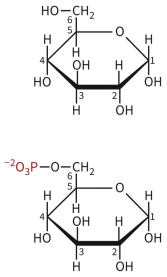
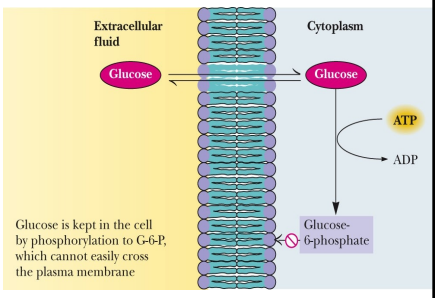
Reactions of Glycolysis Metabolism
Glucokinase / Hexokinase - (GK/HK)







Key glycolytic enzyme

-1st step in glycolysis; *DG* large, negative

-Hexokinase (and glucokinase) act to phosphorylate glucose and keep "trap" Glucose in the cell

-1 ATP is consumed - is considered an irreversable step. Also a key glycolytic step GK is NOT inhibited by G6P

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Reactions of Glycolysis Metabolism
Glucokinase / Hexokinase - (GK/HK)

-Glucokinase in liver, pancreas β cells, hypothalamus and small intestine (maintenance of blood glucose levels and glucose responsive tissues)

-Hexokinase in nearly all tissues - non specific for various hexoses

HK K_m for glucose is 0.1 m*M*; GK K_m for glucose is 10 m*M*.

Blood Glc: normal 4 - 5.5, fasting/sleeping 3.5 mM, post meal 6.5-10 mM
Intracellular glucose ranges from 0.2 – 2 mM depending on cell and state

Therefore - HK has a High affinity for glucose while GK has a Low affinity.

- The function of GK is to remove glucose from the blood following a meal

HK – but not GK is regulated. HK – is allosterically product inhibited.

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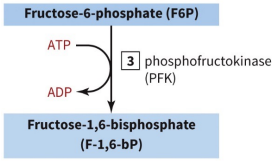
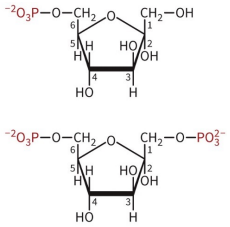
Phosphofructokinase (PFK)

key glycolytic enzyme

-ATP consumed, another phosphoryl transfer (kinase) reaction
-Fructose 1-6- bis phosphate produced

-Rate determining reaction - first commited step in glycolysis
-Commited step with large –ΔG
-Highly regulated





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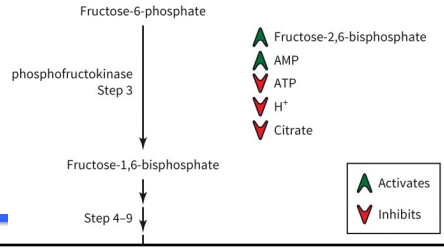
Phosphofructokinase

-Allosterically regulated.

- Homotetramer in mammalian cells.
- Two conformations: Active (R) and Inactive (T)
- TWO ATP Binding sites – active site and regulatory site
- PFK is inhibited by high energy signals, ATP and Citrate. A feedforward and feedback mechansim.
- Activated by low energy signal AMP – which also competitively binds at the regulatory binding site.
- AMP reverses ATP inhibition (thus an activator)
- Fructose-2,6-bisphosphate is strong allosteric activator

Impact of Allosteric Regulation:

- PFK increases activity when energy status is low
- PFK decreases activity when energy status is high



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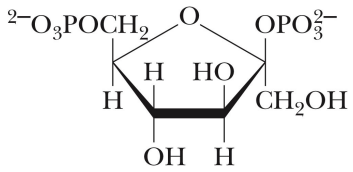
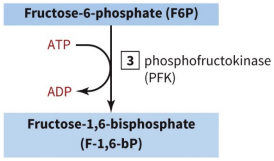
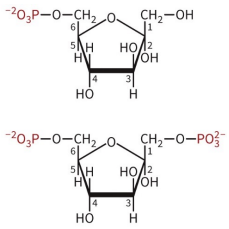
F-2,6-BP regulates Phosphofructokinase



Fructose-2,6-bisphosphate





Phosphofructokinase is regulated by fructose-2,6-bisphosphate, a potent allosteric activator that increases the affinity of phosphofructokinase for the substrate fructose-6-P.

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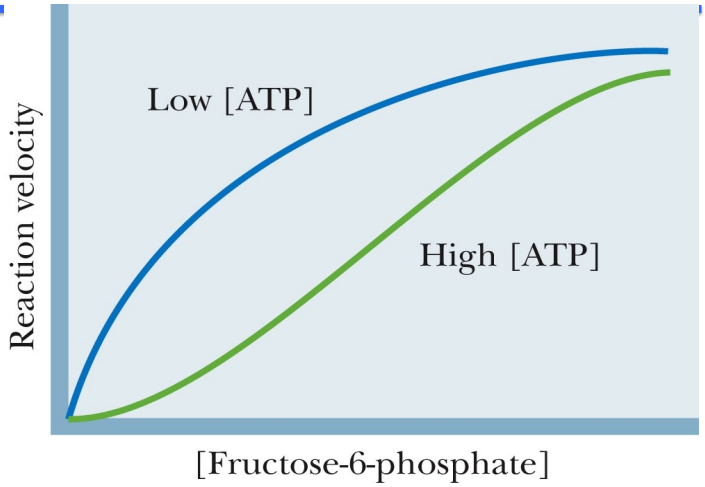
Phosphofructokinase behaves cooperatively at high [ATP]



At high ATP, phosphofructokinase (PFK) behaves cooperatively and the activity plot is sigmoid.

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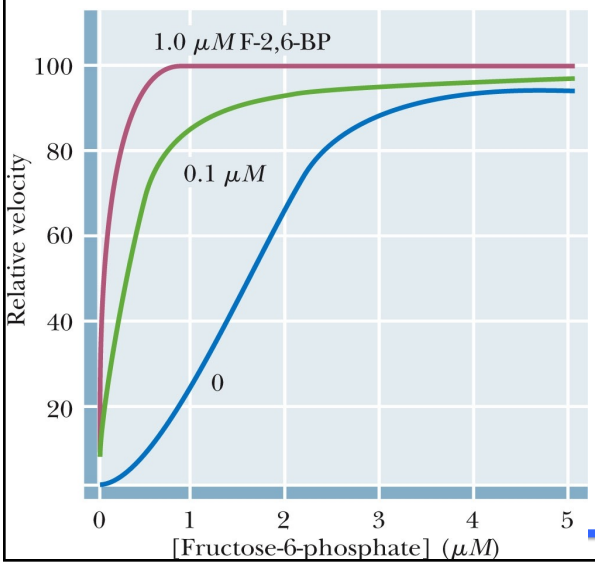
Phosphofructokinase is activated by F-2,6-BP



Fructose-2,6-bisphosphate activates phosphofructokinase, increasing the affinity of the enzyme for fructose-6-phosphate and restoring the hyperbolic dependence of enzyme activity on substrate concentration.

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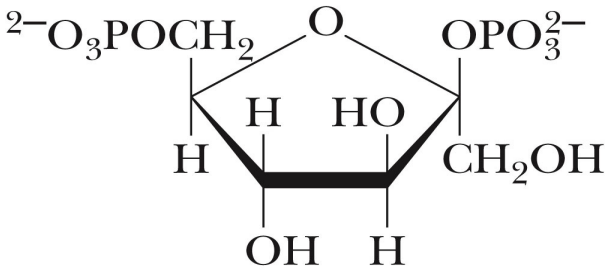
F-2,6-BP regulates Phosphofructokinase



Fructose-2,6-bisphosphate

Phosphofructokinase is regulated by fructose-2,6-bisphosphate, a potent allosteric activator that increases the affinity of phosphofructokinase for the substrate fructose-6-P.

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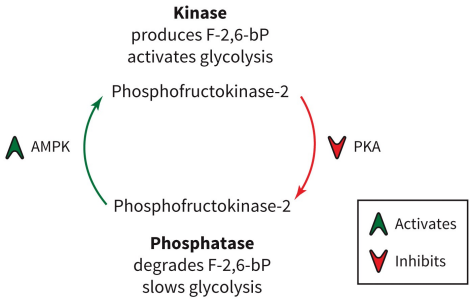
Regulation of PFK-2

Rate-limiting step



Protein Kinase A – Ser/Thr kinase.
cAMP activates PKA
cAMP production and degradation is regulated by hormone action

- Glucagon and epinephrine increase cAMP
- Insulin leads to decrease in [cAMP]

AMP is an allosteric activator of PFK leads to F26bP by activates AMP Kinase (AMPK) further activating PFK

Regulation of phosphofructokinase-2 (PFK-2). PFK-2 has both kinase and phosphatase activities. The activity of PFK-2 is regulated by PKA and AMPK.

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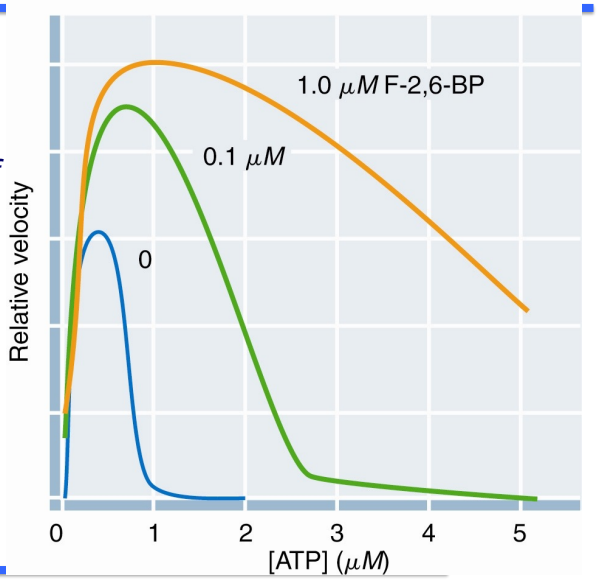
F-2,6-BP reverses the inhibition of PFK by ATP

F-2,6-BP stimulates PFK by **decreasing** *the inhibitory effects of ATP.*



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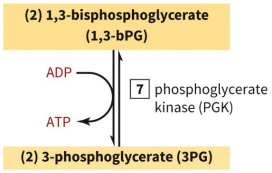
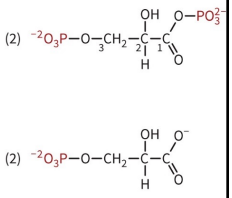
Phosphoglycerate Kinase (PGK)

- A kinase but different from the protein kinases discussed earlier (how so?)
- ATP produced through **substrate level phosphorylation** (O_2 is not involved such as in oxidative phosphorylation)





- The phosphoryl group of C1 of 1,3 BPG is transferred to the beta phosphoryl of ADP
- The active site is closed off from water once the substrate binds similar to the mechanism found with hexokinase
- The formation of 1,3 BPG has a positive standard state free energy change. The reaction can proceed due to thermodynamic coupling with the PGK reacton

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Pyruvate Kinase (PK)

key glycolytic enzyme

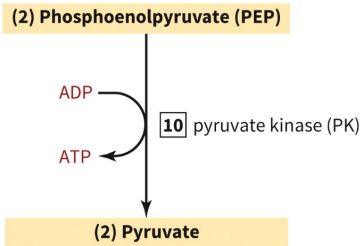
-Substrate level phosphorylation / ATP produced by the release of free energy from PEP

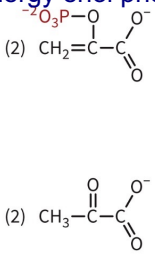
- Committed irreversible step

-Transfer of phosphoryl to ADP produced second ATP

-Spontaneous shift (tautomerize) from enol to keto form yielding pyruvate

-Dehydration of 3PG would not produce a high energy enol phosphate.





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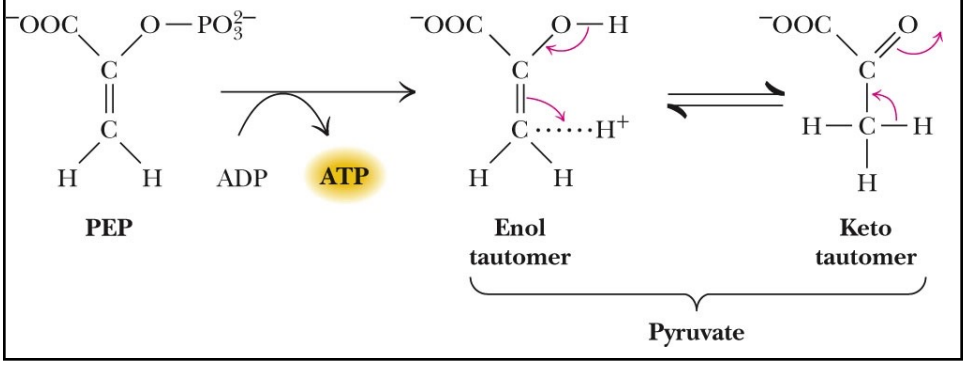
Pyruvate Kinase (PK)

- These two ATP (from one glucose) can be viewed as the "payoff" of glycolysis
- Large, negative *DG* – indicating that this reaction is subject to regulation
- PK is allosterically activated by AMP, F-1,6-bisP
- PK is allosterically inhibited by ATP and acetyl-CoA
- Understand the keto-enol equilibrium of pyruvate; it is the key to understanding the pyruvate kinase reaction



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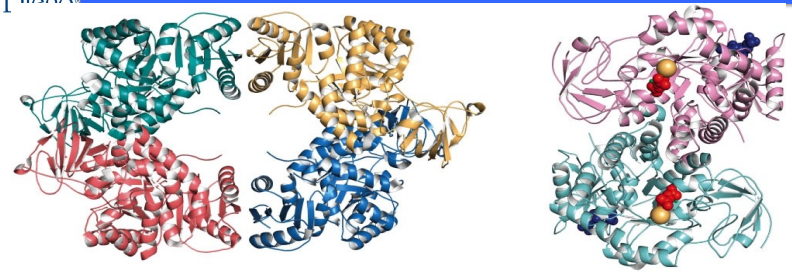
Active as a tetramer



The structure of the pyruvate kinase tetramer is sensitive to bound ligands. Shown are the inactive *E. coli* enzyme in the absence of ligands (left), and the active form of the yeast dimer (right), with fructose-1,6-bisphosphate (an allosteric regulator, blue), substrate analog (red), and K^+ (gold).

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Pyruvate Kinase Regulation

Two different isozymes: L (liver) and M (muscle) Both are tetramers.

- PK is allosterically activated by the substrate PEP
- Feed forward activation by F 16 BP
- ATP and alanine inhibits
- Only the Liver form is phosphorylated at C terminal by PKA (remember cAMP)
- The resulting phosphorylation shifts the enzyme from tetrameter (active) to monomer (inactive) form
- This type of regulation is only observed in liver - why only in the liver and why is that important? Think in terms of energy needs during fight or flight. Muscle want ATP and liver can use other forms of energy sparing glucose for other tissues.

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Pyruvate Kinase Regulation

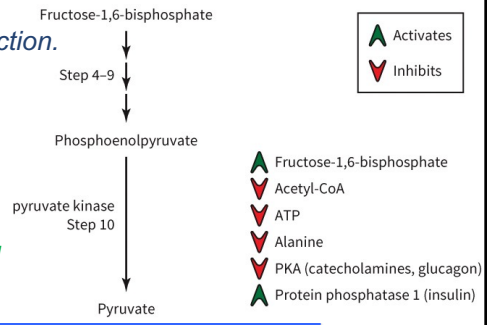
Liver PK – *Liver uses glc for storage and makes glucose in times of need.*

- Inhibited after PKA phosphoryltation
- F-1,6 BP allosteric activator (feed forward – high glc signal)
- Inhibited by ATP, Ala and Acetyl-CoA (all high E signals)

Muscle PK – *Uses Glc for ATP production.*

- Only stimulated by F-1,6 bP
- Not phosphorylated
- Focus on creating ATP

Allows liver to stop using and instead produce Glc while muscles and other tissues continue to use Glc (from liver and glycogen) in low E or high stress states



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Energy of glycolysis

- which reactions are exothermic and endothermic how does this relate to regulation
- total amount of ATP produced by metabolism of glucose to pyruvate - net gain of 2 ATP (fate of NADH depends on cell type and oxidation state of the cell)
- maximum value of 3 ATP can be formed from NADH

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How Do Cells Regulate Glycolysis?

The elegant evidence of regulation

Standard state ΔG values are scattered, with both plus and minus values and no apparent pattern

The plot of ΔG values in cells is revealing:

- Most values near zero
- 3 of 10 reactions have large, negative ΔG

These 3 reactions with large negative ΔG are sites of regulation (HK, PFK, PK)

Regulation of these three reactions can turn glycolysis off and on

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Access to the blood

Glucose Transporters – (GLUT) Influx of glucose into cells

- 5 different transporters
- 12 transmembrane regions

Glut 1 and Glut 3

- Present in all cells
- Low Km, high affinity but have a lower general rate of transport
- Low Km results in a HIGH basal level of entry into cells

Glut 2 Liver and pancreatic cells (insulin release)

- Low affinity, high Km
- Sensitive to blood glucose levels - HOW? - BOTH tissues!
- Sensitivity results in the sparing of glucose by liver for brain and muscles (non gluconeogenic)

Glut 4 Muscles and Fat

- Transport regulated by insulin
- Number of receptors on the cell membrane is altered by endocytosis into intracellular stores
- Main transporter of glucose for these cells

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3 key regulatory enzymes -ΔG steps

1) Hexokinase and Glucokinase (HK/GK)

Glucose + ATP -> Glucose -6 phosphate + ADP

Plays a large part in blood glucose stabilization

Hexokinase is inhibited by formation of product

Glucokinase is not (more sensitive to glucose concentration). Liver contains GK activity - liver is not subjected to inhibition ® liver gets some glucose even if rate of glycolysis is high

Km of glucose of HK << GK -> tissues like brain and muscle get first "shot" at glucose

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The Warburg Effect and Cancer

- Otto Warburg observed in 1924 that rapidly proliferating cancer cells metabolize glucose mainly to lactate, even when O_2 is plentiful
- It has been suggested that this behavior arises because cells need more than ATP – they must synthesize large amounts of nucleotides, amino acids, and lipids
- This requires lots of NADPH for biosynthesis as well as intermediates for building blocks
- Cancer cells divert large amounts of glucose to the pentose phosphate pathway to produce NADPH

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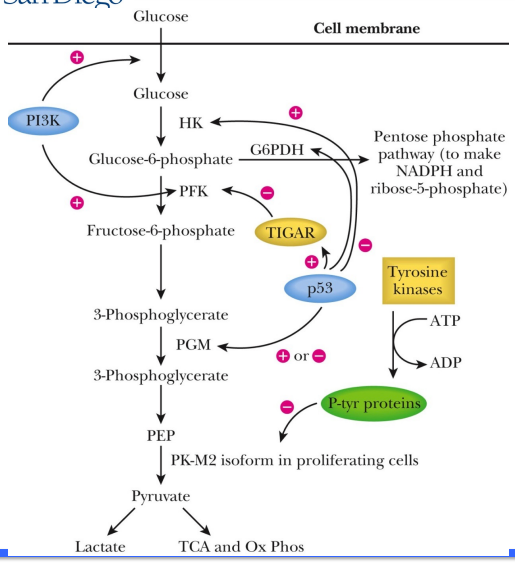
The Warburg Effect and Cancer



Signaling proteins and pathways regulate the glycolytic pathway. Cancer cells route up to 90% of acquired glucose and glutamine into lactate and alanine, producing large amounts of NADPH

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