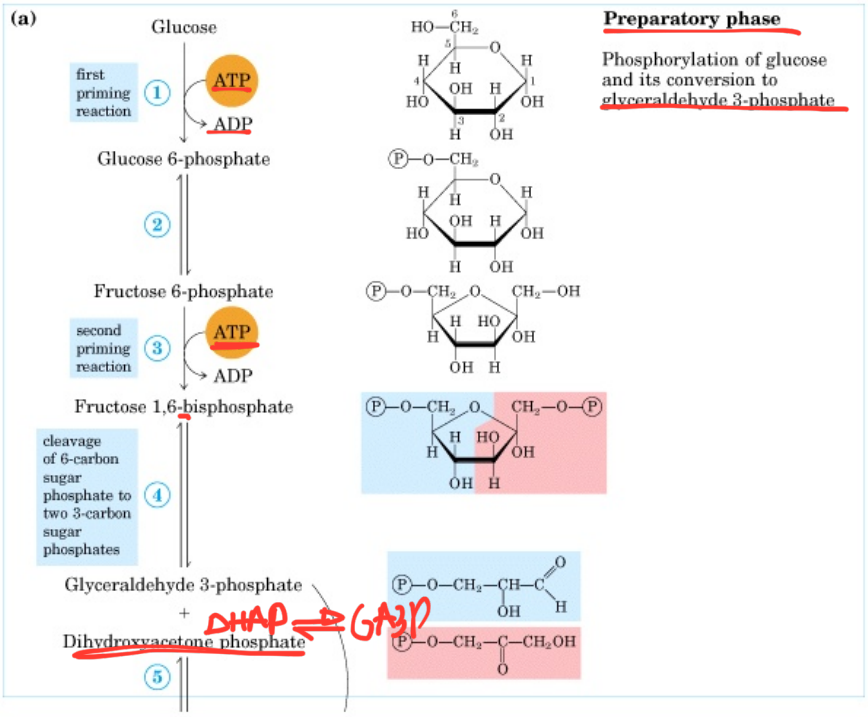
Glycolysis

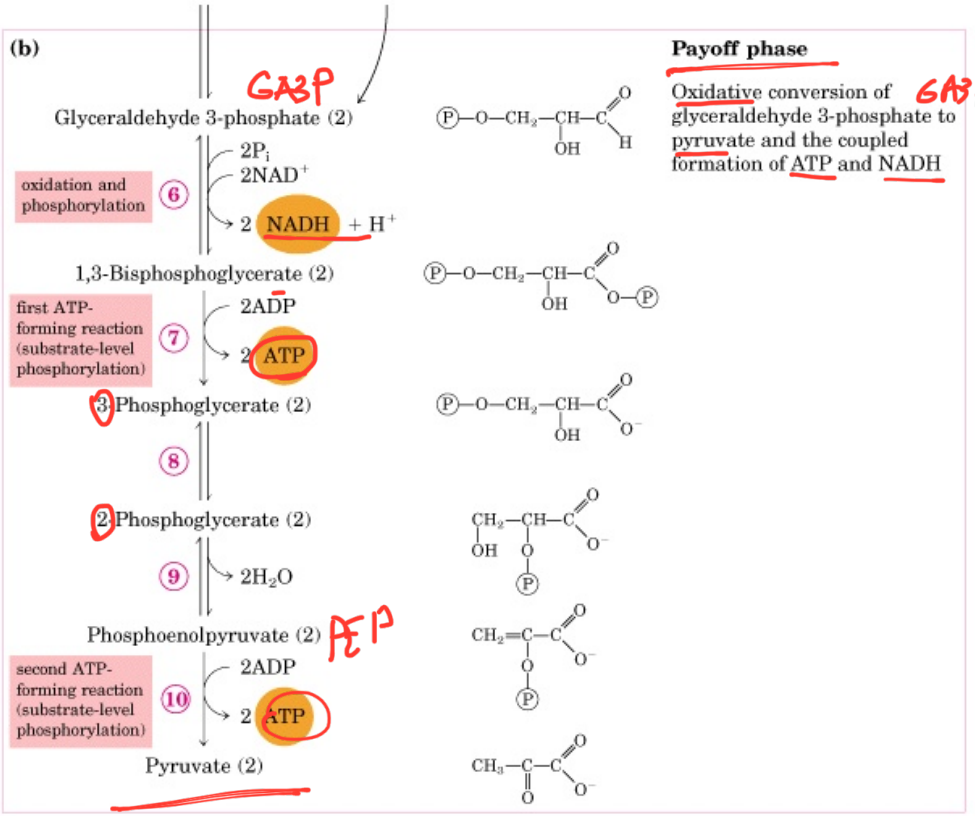
Chapter 3

GLYCOLYSIS

- Pathway that occurs in the cytosol of cell
- During the sequential reactions of glycolysis, some of the free energy released from glucose is con- served in the form of ATP and NADH
- Does not require oxygen
- Active in all tissues
- Impt for ATP production specially when oxygen is depleted, or when cells lack mitochondria
- 10 step process
- 3 irreversible enzymatic steps
- **2 stages**

- The conversion of glucose to pyruvate is not very efficient; in other words, there is still a lot of energy remaining in the pyruvate molecule.
- From pyruvate, the metabolic course depends largely on the availability of oxygen and reducing units within the cell. When oxygen is lacking, pyruvate is converted to lactate. When sufficient oxygen is present, glucose is catabolized more efficiently in mitochondria to carbon dioxide and water.
- Under aerobic conditions, pyruvate can be transported into the mitochondria and participates in the TCA cycle, in which it becomes completely oxidized to CO2 and H2O.
- Complete oxidation is accompanied by the release of relatively large amounts of energy, most of which is captured in ATP molecules by the mechanism of oxidative phosphorylation





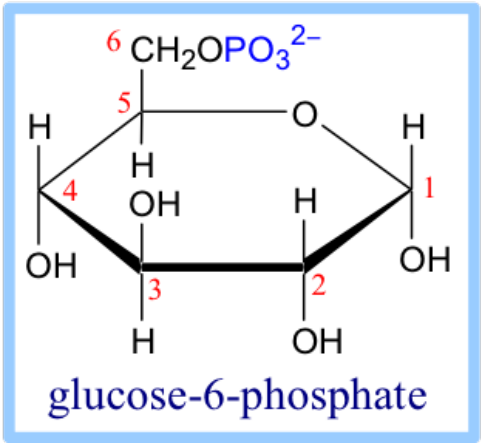
Stages of Glycolysis

- **Stage 1: (reactions 1-5) A preparatory stage in which glucose is phosphorylated, converted to fructose which is again phosphorylated and cleaved into two molecules of glyceraldehyde-3-phosphate. In this phase there is an investment of two molecules of ATP.**

- **Stage 2: (reactions 6-10) The two molecules of glyceraldehyde-3-phosphate are converted to pyruvate with concomitant generation of four ATP molecules and two molecules of NADH. Thus, there is a net gain of two ATP molecules per molecule of glucose in glycolysis.**

Importance of phosphorylated intermediates:

- Plasma membrane generally lacks transporters for phosphorylated sugars, the phosphorylated glycolytic intermediates cannot leave the cell.
- **Conservation of free energy in high energy phosphate bond.**



glucose-6-phosphate

- Influx of glucose continues until transporters are saturated
- Glucose is trapped in cell

GLYCOLYSIS

- Step 1
  - Phosphorylation of glucose using *hexokinase* glucose 6 phosphate using up one ATP molecules
  - Irreversible step
  - Regulated by glucose 6 phosphate, acts as a negative feedback regulator
  - Trapping of glucose inside cell, addition of a negative polar group

Hexokinase is present in most cells.

It requires Mg-ATP complex as substrate. Uncomplexed ATP is a potent competitive inhibitor of this enzyme.

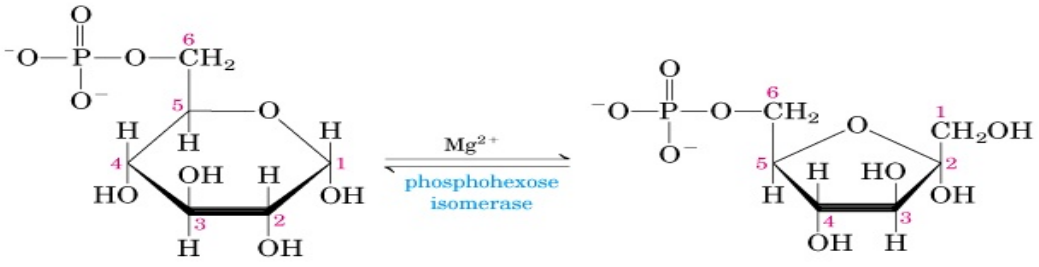
Enzyme catalyzes the reaction by proximity effect; bringing the two substrate in close proximity.

I. **This enzyme undergoes large conformational change upon binding with Glucose. It is inhibited allosterically by G6P**

Isomerization of G6P to Fructose 6 phosphate

Step 2

- **Phosphohexose isomerase (phospho- glucose isomerase)** catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to **fructose 6-phosphate,** a ketose:



Glucose 6-phosphate

Fructose 6-phosphate

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

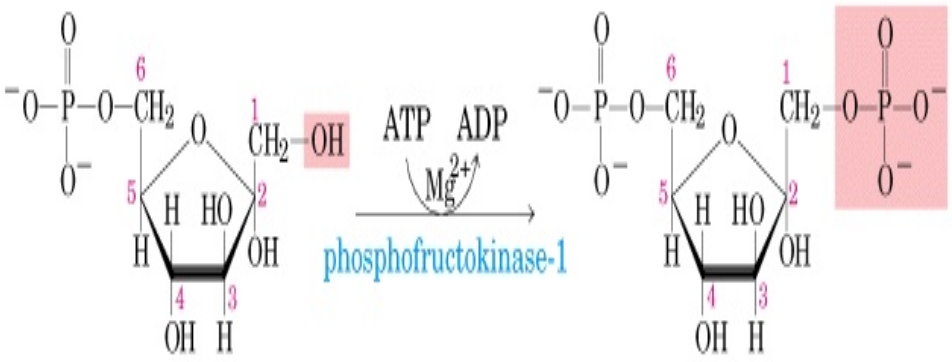
Step 3

- ATP is consumed
- Irreversible step
- Highly regulated step

- **Transfer of phosphoryl group from ATP to C-1 of F6P to produce Fructose 1,6 bisphosphate.**

ATP is an allosteric inhibitor, and Fructose 2,6 bisphosphate is an activator of this enzyme.

ADP and AMP also activate PFK-1 whereas



Fructose 6-phosphate

Fructose 1,6-bisphosphate

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

Fructose 6P → Fructose-1,6-P

↑ATP → stop glycolysis [F2,6BP] → ↑[F1,6BP] → glc

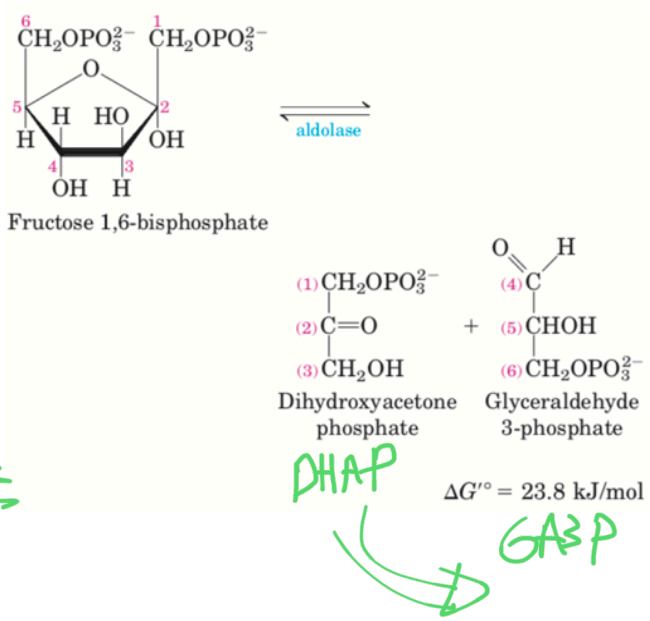
ATP → ADP + Pi

↑ADP → start glycolysis

Step 4

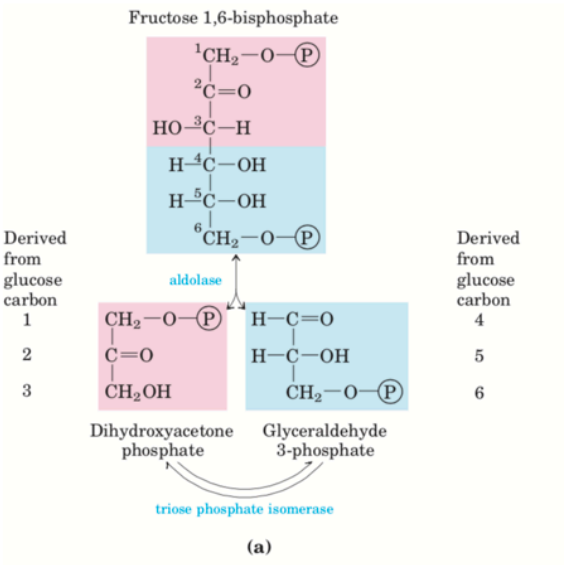
- Cleavage of Fructose 1,6-Bisphosphate The enzyme fructose 1,6-bisphosphate aldolase catalyzes a reversible aldol condensation

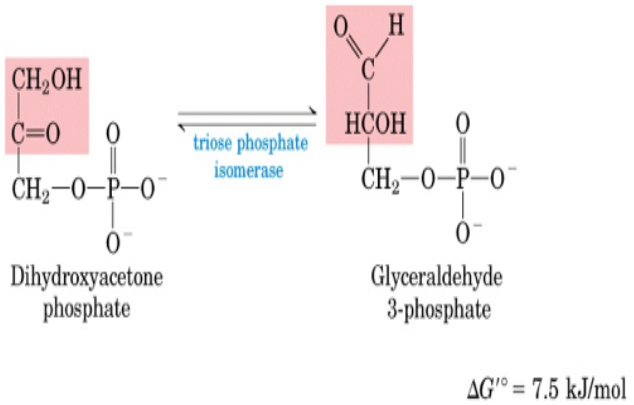
- **The standard free energy change is positive in the forward direction, meaning it requires energy. Since the product of this reaction are depleted very fast in the cells, this reaction is driven in forward direction by the later two reactions**



Step 5

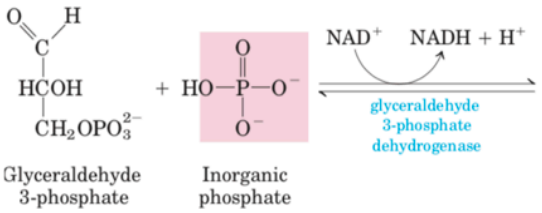
Triose phosphate isomerase reaction: Conversion of Dihydroxyacetone phosphate to glyceraldehyde 3 Phosphate.

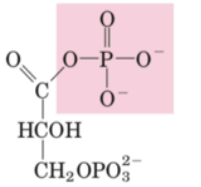




Step 6

Glyceraldehyde-3-phosphate dehydrogenase reaction (GAPDH): Conversion of GAP to Bisphosphoglycerate





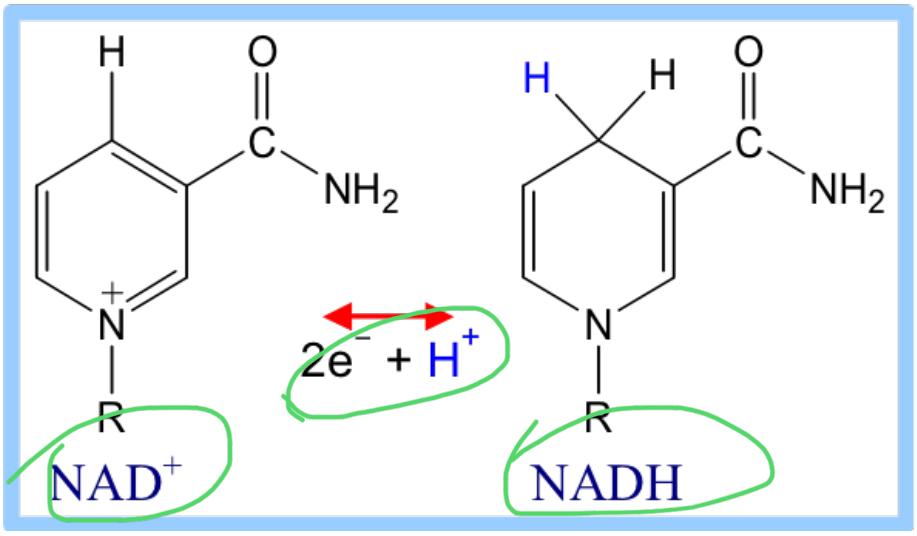
1,3-Bisphosphoglycerate

$\Delta G'^{\circ} = 6.3$ kJ/mol

I. **The Payoff Phase of Glycolysis Yielding ATP and NADH**

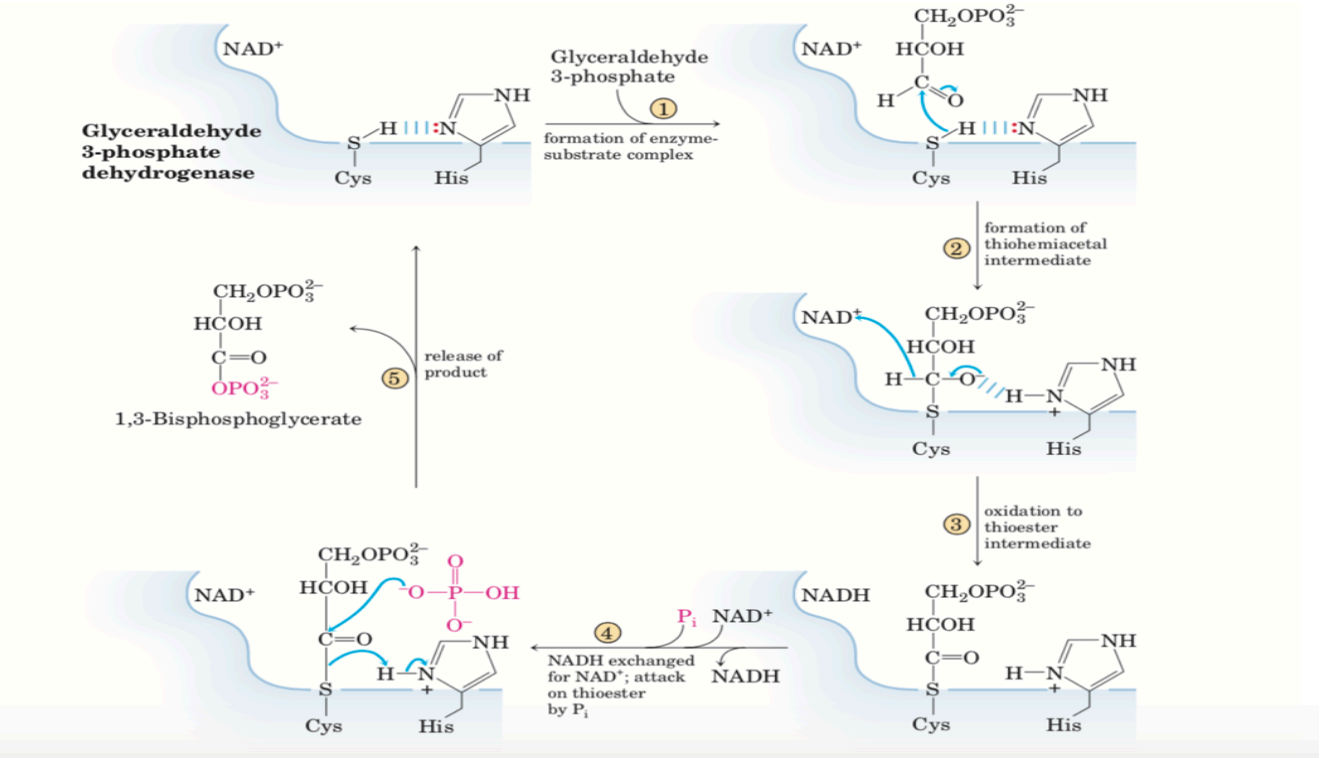
II. **An inorganic phosphate is incorporated in this reaction without any expense of ATP.**

III. **NAD+ is the cofactor in this reaction which acts as an oxidizing agent**



Oxidation- reduction reaction

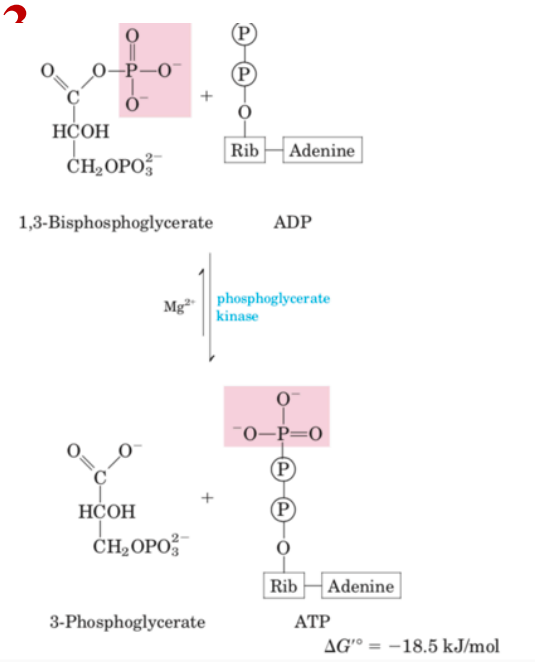
- Glyceraldehyde 3-phosphate is covalently bound to the dehydrogenase during the reaction
- The aldehyde group of glyceraldehyde 3-phosphate reacts with the OSH group of an essential Cys residue in the active site
- **Nucleophilic attack by SH group on aldehyde group forming a thiohemiacytal**
- **Direct transfer of hydride to NAD+ leading to the formation of thioester. Energy of this oxidation is conserved in synthesis of thioester and NADH**
- **Nucleophilic attack on thioester by PO4– - to form 1,3 bisphosphoglycerate**



Phosphoryl Transfer fro... 1,3 Bisphosphoglycerate to A...

Step 7

Enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3- phosphoglycerate:**



This reaction and the 6th step are coupled reaction generating ATP from the energy released by oxidation of 3-phosphoglyceraldehyde

- **Step 6** Endergonic → Absorp E Product contain MORE E than the reactants
- **Step 7** Highly exergonic → Releas E

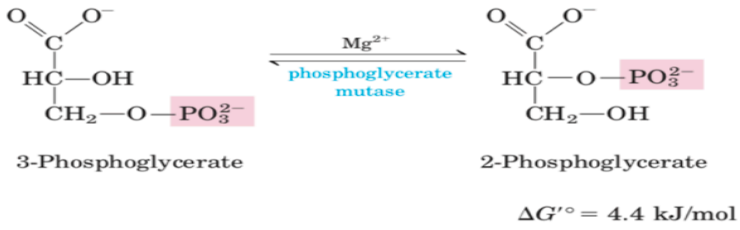
Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

Step 8

The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate;

Mg+2 is essential for this reaction

transfer of the phosphoryl group from enzyme to 3-PG generating enzyme bound 2,3-biphosphoglycerate intermediate.

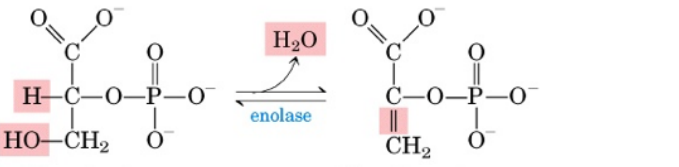


Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

Step 9

Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

generating a compound with high phosphoryl group transfer potential removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP):**

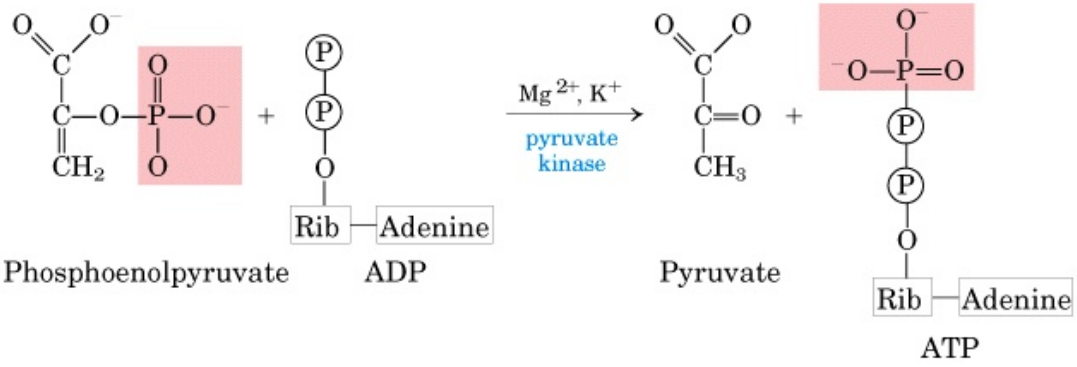


$\Delta G'^{\circ} = 7.5$ kJ/mol

Pyruvate Kinase Reaction: Transfer of phosphoryl group from PEP to ADP generating ATP and Pyruvate

STEP 10

This enzyme requires Mg++ and K+



$\Delta G'^{\circ} = -31.4$ kJ/mol

Balance sheet

• ATP ~P bonds expended =2

• many ~P bonds of ATP produced? 4

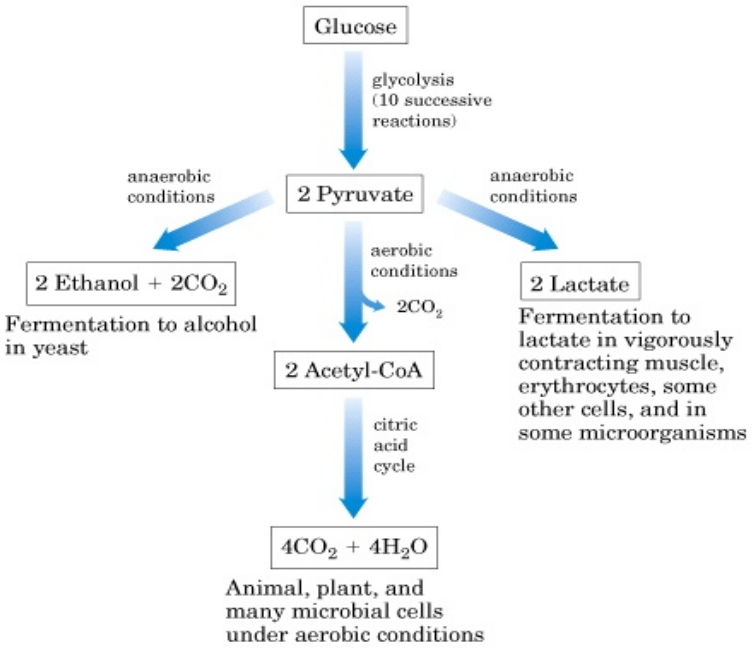
Net production of ~**P** bonds of ATP per glucose= 2

glucose + 2 NAD+ + 2 ADP + 2 Pi □ 2 pyruvate + 2 NADH + 2 ATP

In **aerobic organisms**:

pyruvate produced in Glycolysis is oxidized to CO2 via Krebs Cycle

NADH produced in Glycolysis & Krebs Cycle is reoxidized via the respiratory chain, with production of more ATP.

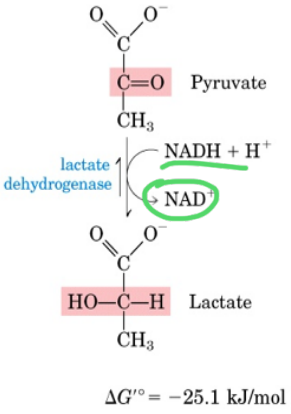


Lactic Fermentation:

Lactic acid fermentation converts the 3-carbon pyruvate to the 3-carbon lactic acid by action of **Lactate dehydrogenase (LDH)**

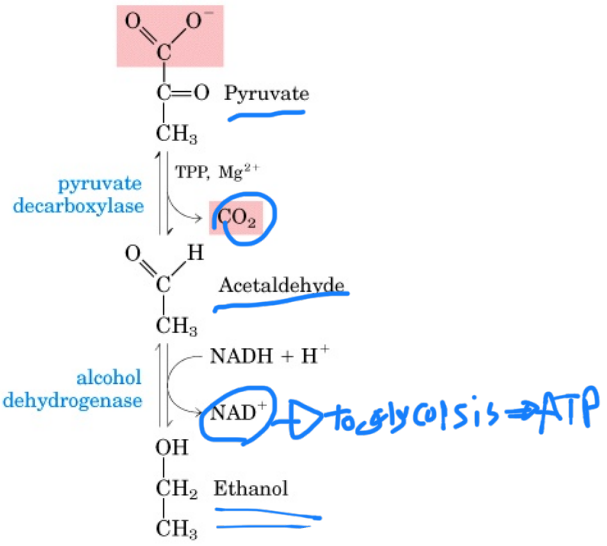
regenerates NAD+ Allowing glycolysis to continue to make ATP in low-oxygen conditions.
Since there is a limited supply of NAD++ available in any given cell, this electron acceptor must be regenerated to allow ATP production to continue

product lactic acid brings the pH lower and causes fatigue



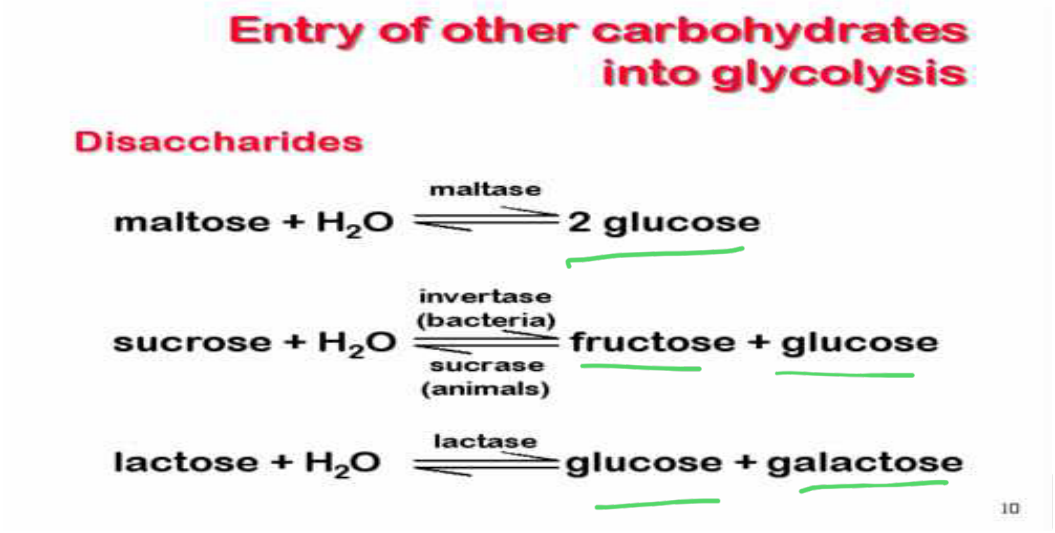
Alcoholic fermentation:

- **Microorganisms and yeast convert pyruvate to alcohol and carbon dioxide to regenerate NAD+ for glycolysis (step 6, GAPDH).**
- Since there is a limited supply of NAD++ available in any given cell, this electron acceptor must be regenerated to allow ATP production to continue.
- **two step process:**
- **This enzyme is Mg++-dependent and requires an enzyme-bound cofactor, thiamine pyrophosphate (TPP).**



Feeder Pathways for Glycolysis

• Starch and glycogen- disaccharides



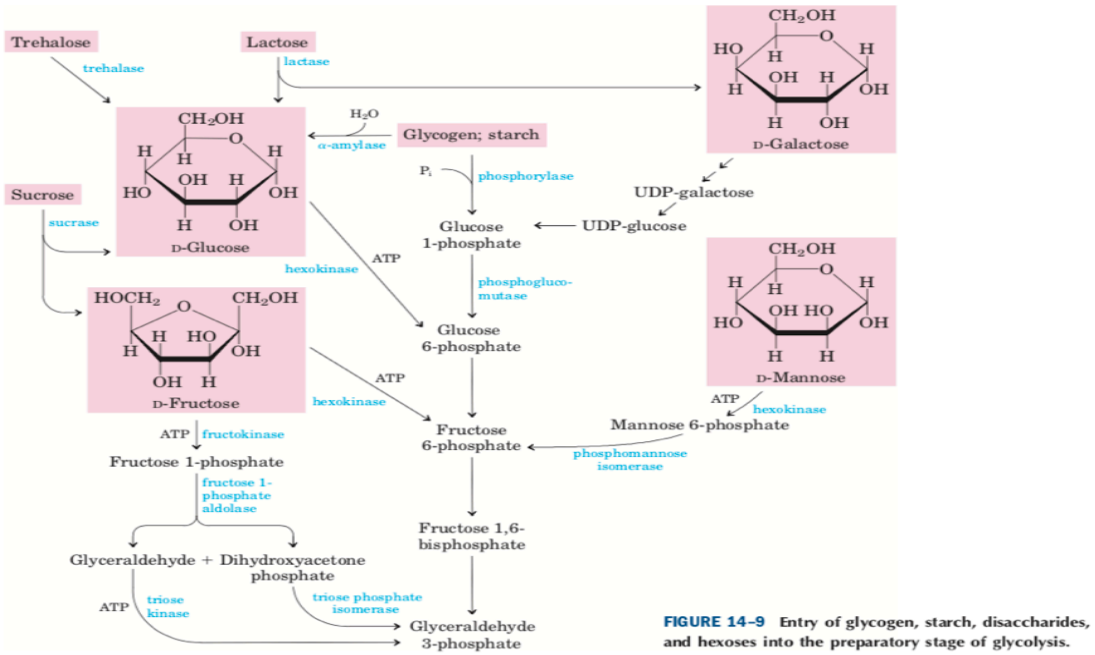


FIGURE 14–9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

Fructose

Fructose enters glycolysis

- **In skeletal muscle:** the glycolytic enzyme, hexokinase will convert fructose to fructose-6-phosphate
  - Low affinity to fructose
- **Liver cells** have another enzyme fructokinase(Abundant in liver)
  - Strong affinity to fructose
  - Makes fructose 1 – phosphate
  - An additional cleavage and phosphorylation is necessary
    - The six-carbon fructose-1-phosphate molecule is split into three-carbon molecules by fructose-1-phosphate aldolase. The products are glyceraldehyde and dihydroxyacetone phosphate
    - Triose kinase phosphorylates glyceraldehyde at C-3 using an ATP. The product can now enter glycolysis as glyceraldehyde-3-phosphate
    - Dihydroxyacetone phosphate ?
    - Fructose G3P, fructose in the liver follows a one- way trip to becoming pyruvate following a high CHO meal

Fructose

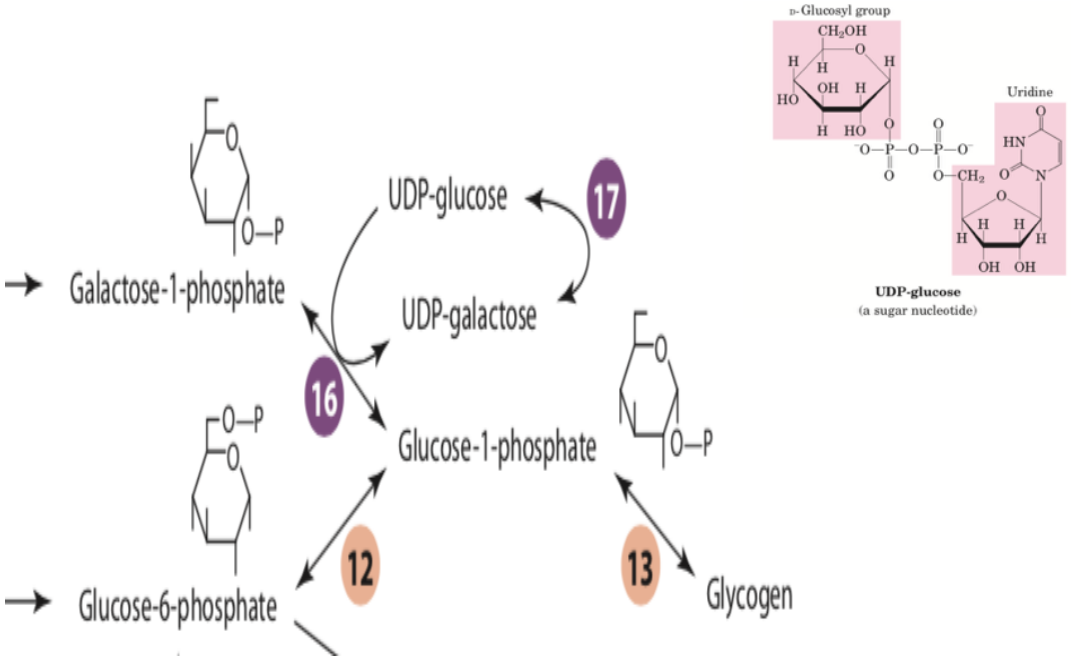
- In contrast to glucose, which circulates throughout the body, fructose is metabolized primarily by the liver when first absorbed from the gastrointestinal tract (Primarily by fructokinase)

In Non liver cells

- Fructose + ATP $\xrightarrow{\text{Hexokinase}}$ Fructose-6-phosphate + ADP
- Fructose 6-p is fed into glycolysis

GALACTOSE

- Galactose is immediately phosphorylated upon entering the cell
- Occurs in liver when galactose is first absorbed from the gastrointestinal tract. The reaction is catalyzed by galactokinase and produces galactose-1-phosphate.
- *Galactose-glucose interconversion pathway*
- The fate of glucose-1-phosphate from galactose depends on the energy status of the cell.
- High energy, high glucose concentration , the glucose- 1-phosphate from galactose is driven mostly toward glycogenesis



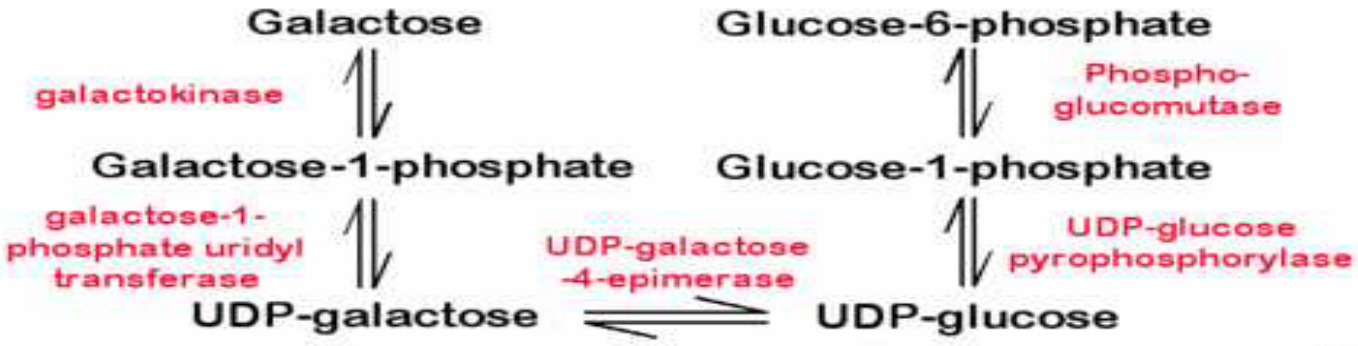
Galactose

- First, the galactose is phosphorylated by galactokinase to yield galactose-1-phosphate
- transfer of UDP from uridine diphosphoglucose (UDP-glucose) catalyzed by galactose-1-phosphate uridyltransferase (GALT)
- The GALT-catalyzed reaction generates UDP-galactose and Glucose-1- phosphate
- The UDP-galactose is epimerized to UDP-glucose by UDP-galactose-4 epimerase (GALE)
- glucose-1-phosphate is converted to G6P by phosphoglucose mutase

Entry of other carbohydrates into glycolysis

Galactose

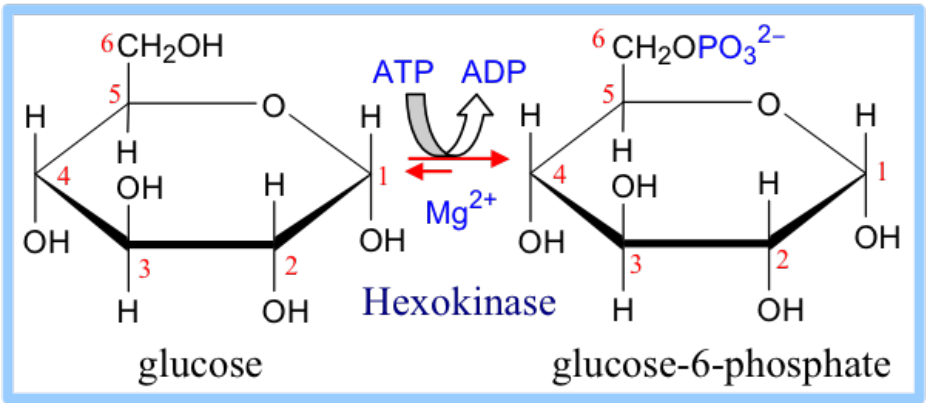
Five reactions are required to transform it into glucose-6-phosphate.



Regulation of Glycolysis:

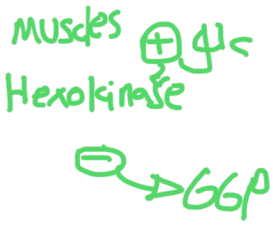
- **Substrate limited : When concentrations of reactants and products in the cell are near equilibrium, then it is the availability of substrate which decides the rate of reaction.**
- **Enzyme limited: When concentration of substrate and products are far away from the equilibrium, then it is the activity of enzyme that decides the rate of reaction**
- Three reactions of glycolysis are so exergonic as to be essentially irreversible: those catalyzed by hexokinase, PFK-1, and pyruvate kinase

- Hexokinase is regulated by the **energy charge** of the cell
- Glucose-6-phosphate inhibits by **competition** at the active site, as well as by **allosteric** interactions at a separate site on the enzyme



Glucose are trapped inside cells through action of hexokinase.. Thus Inhibition will prevent accumulation of glucose from blood into cell

- In Muscle cells, hexokinases are allosterically inhibited by their product, glucose 6-phosphate
- Hexokinase is feedback-inhibited by its product, so the phosphorylation of glucose is inhibited if there is a buildup of glucose-6-p



- Muscle and liver have different functions
- muscle consumes glucose, using it for energy production, whereas liver maintains blood glucose homeostasis by removing or producing glucose
- In Liver, we have Glucokinase
- the glucose concentration at which hexokinase IV is half- saturated (about 10 mM) is higher than the usual concentration of glucose in the blood
- When the blood glucose concentration is high, as it is after a meal rich in carbohydrates, excess glucose is transported into hepatocytes, where hexokinase Converts it to glucose 6-phosphate
- its activity continues to increase as the glucose concentration rises to 10 mM or more.

- Glucokinase is **not** subject to product inhibition by glucose-6-phosphate.
- Liver will take up & phosphorylate glucose even when liver [glucose-6-phosphate] is high
- Glucokinase is subject to inhibition by the reversible binding of a regulatory protein specific to liver
- binding is much tighter in the presence of the allosteric effector fructose 6-phosphate
- During a fast, when blood glucose drops below 5 mM, fructose 6-phosphate triggers the inhibition of hexokinase IV by the regulatory protein, so the liver does not compete with other organs for the scarce glucose.

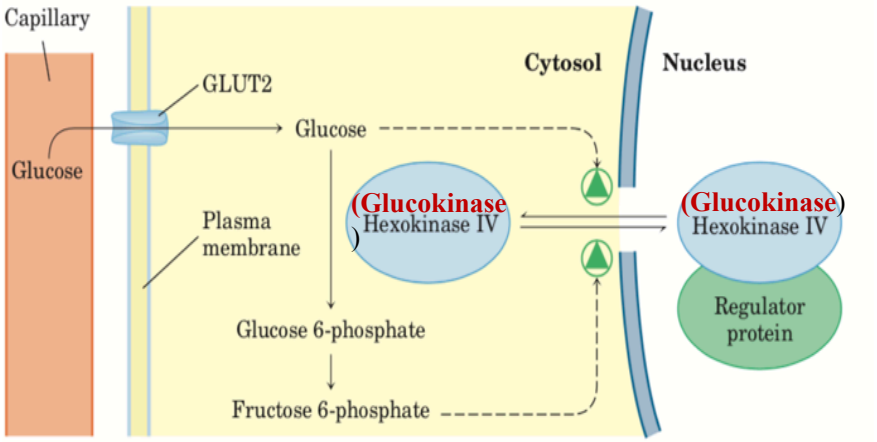
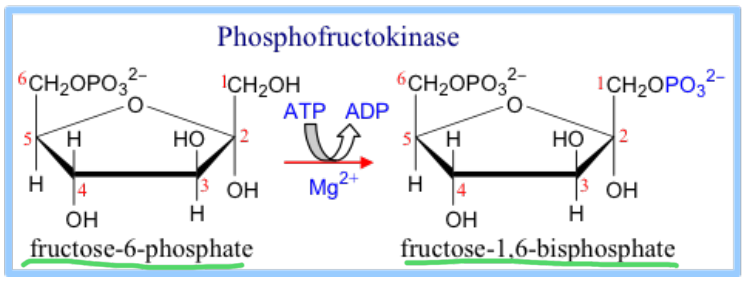


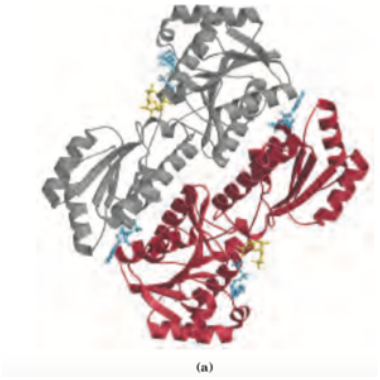
FIGURE 15-17 **Regulation of hexokinase IV (glucokinase) by sequestration in the nucleus.** The protein inhibitor of hexokinase IV is a nuclear binding protein that draws hexokinase IV into the nucleus when the fructose 6-0phosphate concentration in liver is high and releases it to the cytosol when the glucose concentration is high.

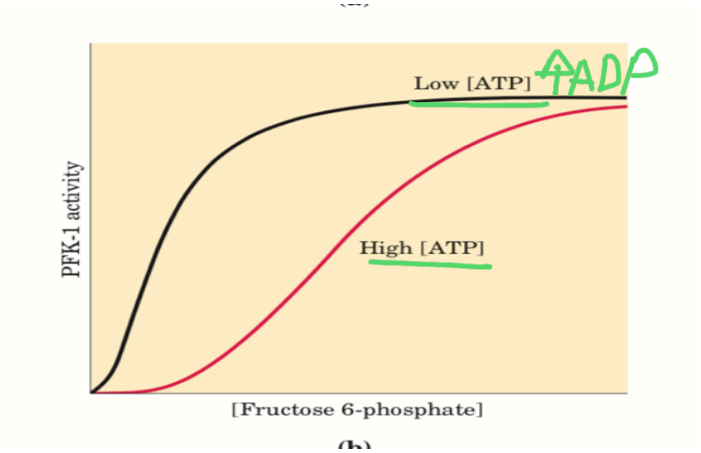
The regulator protein anchors glucokinase inside the nucleus, where it is segregated from the other enzymes of glycolysis in the cytosol
When the glucose concentration in the cell rises, it equilibrates with glucose in the nucleus
Glucose causes dissociation of the regulatory protein, and glucokinase enters the cytosol and begins to phosphorylate glucose.

Phosphofructokinase

- substrate-binding sites and several regulatory sites at which allosteric activators or inhibitors bind.
- **At low concentration, the substrate ATP binds only at the active site.**
- **At high concentration, ATP binds also at a low-affinity regulatory site, promoting the tense conformation**





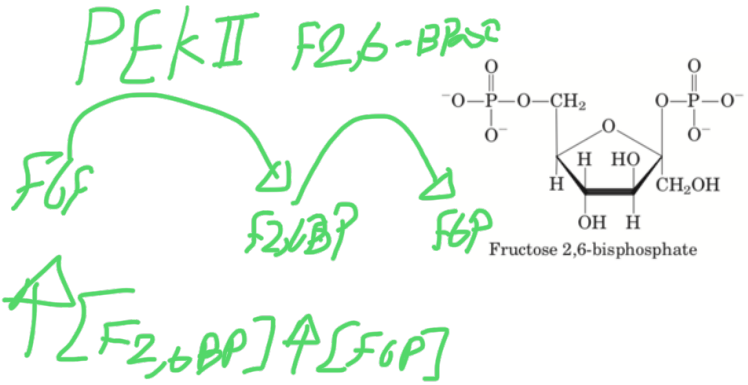


The **tense** conformation of PFK, at **high [ATP]**, has lower affinity for the other substrate, fructose-6-P
AMP, present at significant levels only when there is extensive ATP hydrolysis, relieves inhibition effects of high ATP

- Citrate (the ionized form of citric acid), a key intermediate in the aerobic oxidation of pyruvate, fatty acids, and amino acids, also serves as an allosteric regulator of PFK-1
- high citrate concentration increases the inhibitory effect of ATP, further reducing the flow of glucose through glycolysis

- Significant allosteric regulator of PFK-1 is fructose 2,6-bisphosphate,
- When fructose 2,6-bisphosphate binds to its allosteric site on PFK-1
  - Increases that enzyme's affinity for its substrate fructose 6-phosphate
  - Reduces its affinity for the allosteric inhibitors ATP and citrate

fructose 2,6-bisphosphate is a *regulator* whose cellular level reflects the level of glucagon in the blood, which rises when blood glucose falls.

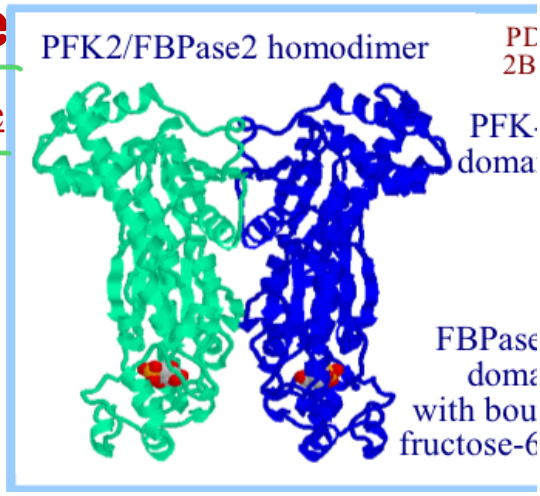


Fructose 2,6-bisphosphate

only found in Liver

Formed by phosphorylation of fructose 6-phosphate

- catalyzed by **phosphofructokinase-2 (PFK-2)**
  - **Fructose-6-phosphate + ATP □ fructose-2,6-bisphosphate + ADP**



Broken down by fructose 2,6- bisphosphatase (FBPase-2)

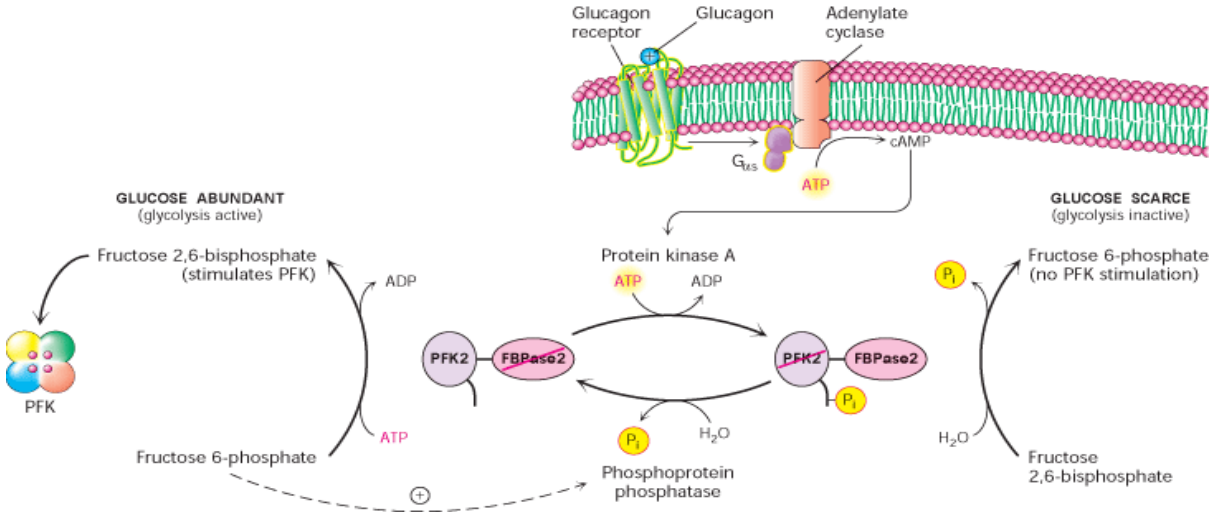
- **Fructose-2,6-bisphosphate + H2O □ fructose-6-phosphate + Pi**

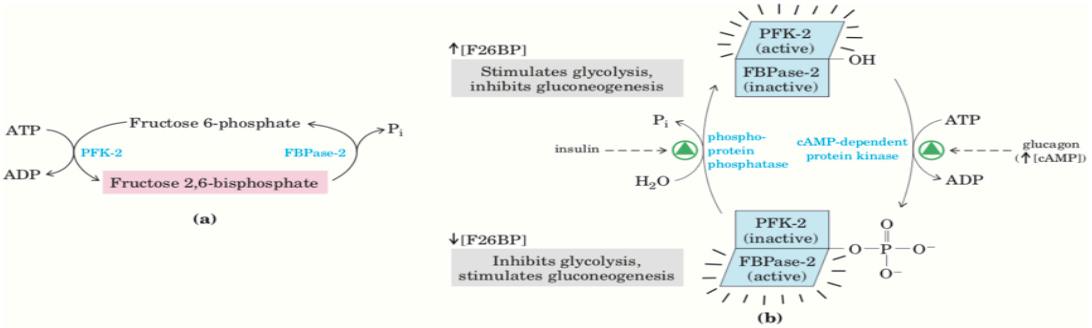
- **PFK-2** and **FBPase-2** are two distinct enzymatic activities of a single, bifunctional protein

Glucagon and Insulin Regulate Activity of the two different domains□ concentration of fructose 2,6- bisphosphate in cell

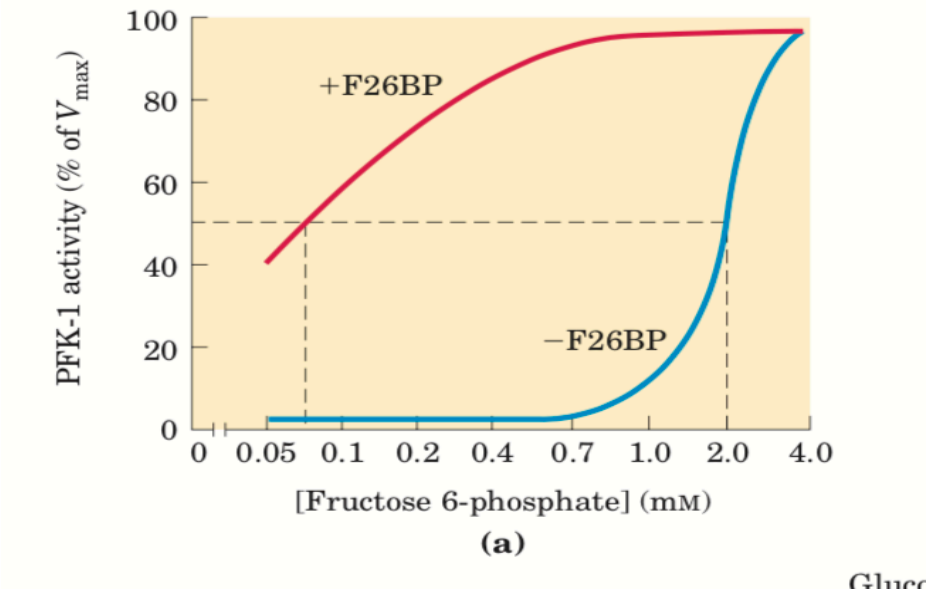
Glucagon secretd from pancrease, binds to cell memebrane receptor

- stimulates the adenylyl cyclase of liver to synthesize (cAMP) from ATP
- cAMP activates cAMP-dependent protein kinase which transfers a phosphoryl group from ATP to the bifunctional protein PFK-2/FBPase-2. Phosphorylation of this protein enhances its FBPase-2 activity and inhibits its PFK-2 activity
- Glucagon lowers the cellular level of fructose 2,6-bisphosphate, inhibiting glycolysis and stimulating gluconeogenesis



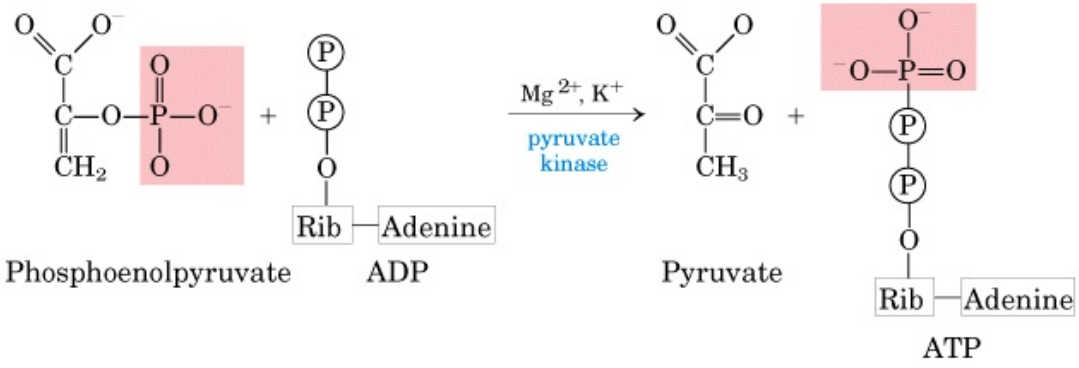


Insulin has the opposite effect, stimulating the activity of a phosphoprotein phosphatase that catalyzes removal of the phosphoryl group from the bifunctional protein PFK-2/FBPase-2.



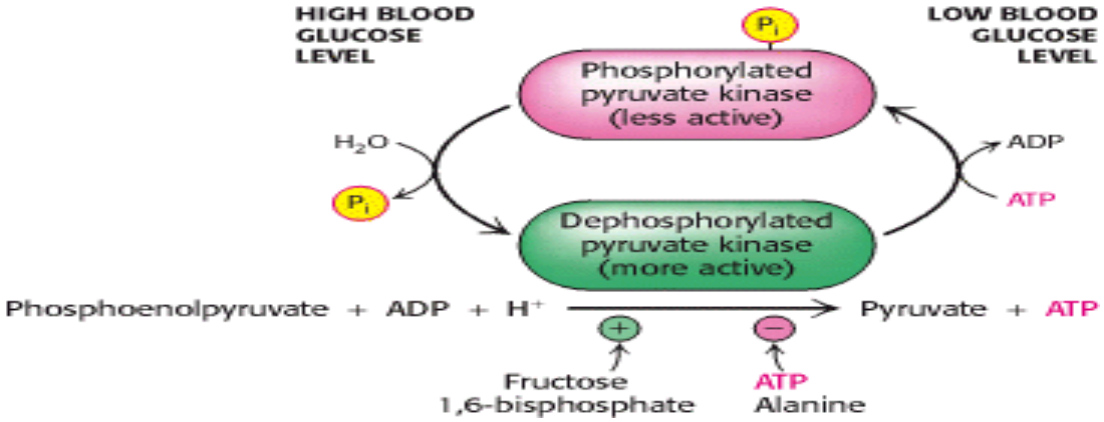
Pyruvate Kinase

Step 10



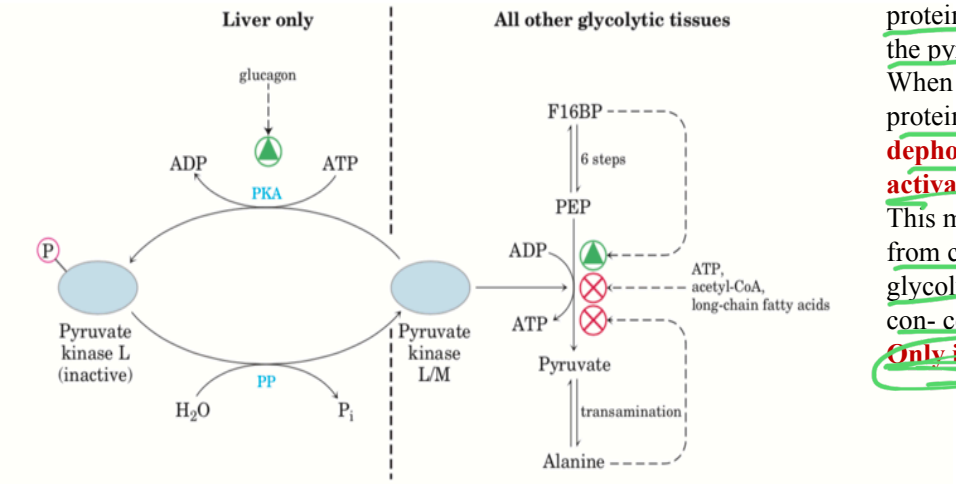
$\Delta G'^{\circ} = -31.4$ kJ/mol

- High concentrations of ATP, acetyl-CoA, and long-chain fatty acids (signs of abundant energy supply) allosterically inhibit pyruvate kinase



- The enzyme (**Pyruvate Kinase)** is allosterically inhibited by ATP, acetyl-CoA, and long-chain fatty acids (all signs of an abundant energy supply), and the accumulation of fructose 1,6-bisphosphate triggers its activation. Accumulation of alanine, which can be synthesized from pyruvate in one step, allosterically inhibits pyruvate kinase, slowing the production of pyruvate by glycolysis

- In liver, also hormonal regulation by glucagon



glucagon activates cAMP-dependent protein kinase which phosphorylates the pyruvate kinase **inactivating it.** When the glucagon level drops, a protein phosphatase **(PP) dephosphorylates pyruvate kinase, activating it.** This mechanism prevents the liver from consuming glucose by glycolysis when the blood glucose con- centration is low

~~**Only in Liver**~~

- **Local control** involves dependence of enzyme-catalyzed reactions on concentrations of pathway substrates or intermediates within a cell.
- **Global control** involves hormone-activated production of second messengers that regulate cellular reactions for the benefit of the organism as a whole.