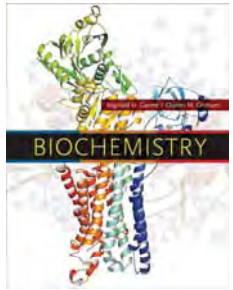
Chapter 18



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Glycolysis

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Before the class…

- Do you know….
  - How glucoses are used in a cell?
  - Is all kinds of cells used glucose in the same way?
  - How many ATP could produce from a glucose?

Outline

- Part 1
  - What Are the Essential Features of Glycolysis?
  - Why Are Coupled Reactions Important in Glycolysis?
  - What Are the Chemical Principles and Features of the First Phase of Glycolysis?
- Part 2
  - What Are the Chemical Principles and Features of the Second Phase of Glycolysis?
- Part 3
  - What Are the Metabolic Fates of NADH and Pyruvate Produced in Glycolysis?
  - How Do Cells Regulate Glycolysis?
  - Are Substrates Other Than Glucose Used in Glycolysis?

Before the class

- Review of Free Energy

$$A + B \rightleftharpoons C + D$$

$$\Delta G = \Delta G^0 + RT \ln \frac{[C]\,[D]}{[A]\,[B]}$$

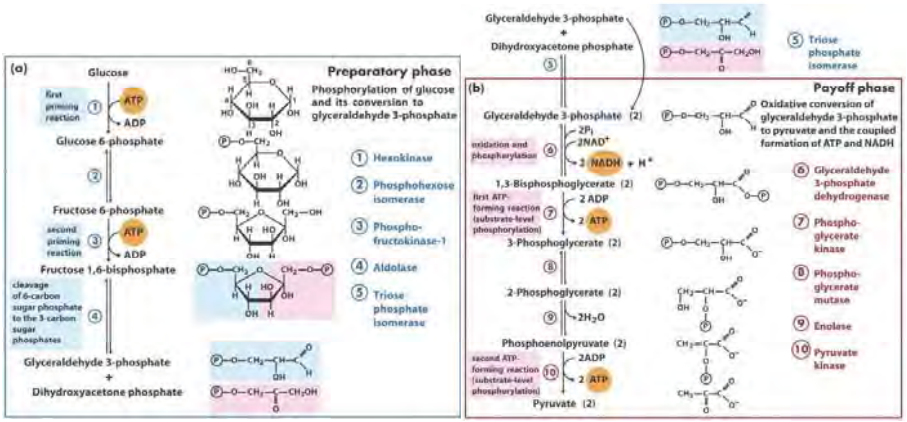
$\Delta G > 0$ *endogenic*; $\Delta G < 0$ *exogenic*

18.1 – What Are the Essential Features of Glycolysis?

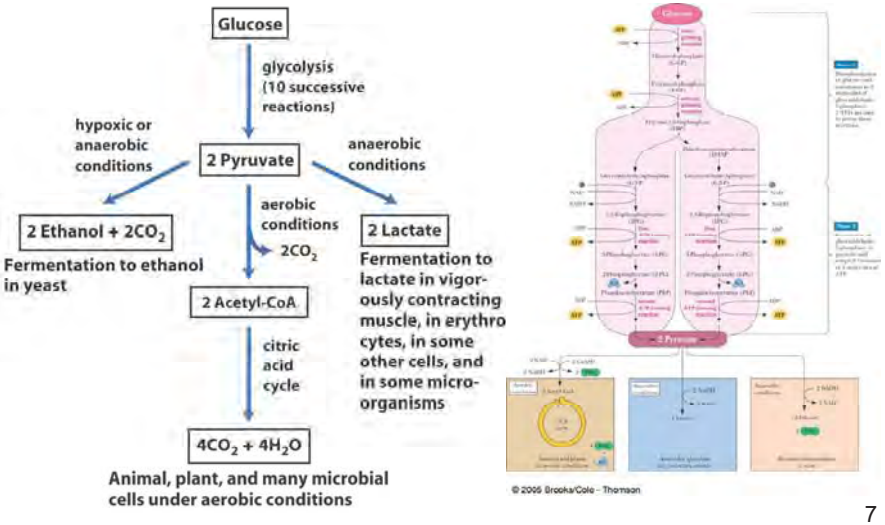
The Embden-Meyerhof (Warburg) Pathway

- Essentially all cells carry out glycolysis
- Ten reactions - same in all cells - but rates differ (some enzyme different!)
- Two phases:
  - First phase converts glucose to two G-3-P
    (preparatory phase: first 5 reactions)
  - Second phase produces two pyruvates
    (payoff phase: last 5 reactions)
- Products are pyruvate, ATP and NADH
- Three possible fates for pyruvate

2 Phases, 10 Steps



3 fates of Pyruvate



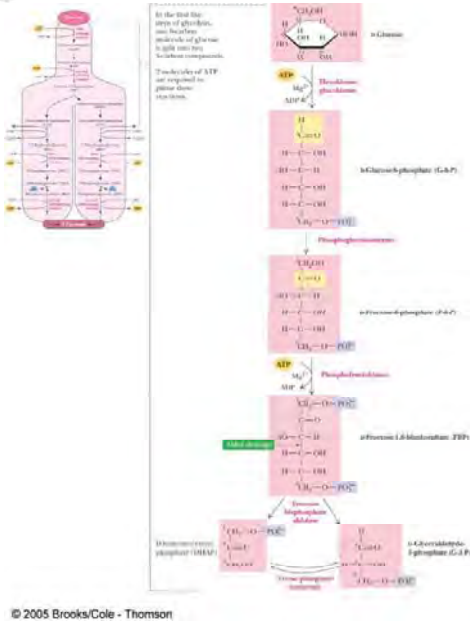
18.2 – Why Are Coupled Reactions Important in Glycolysis?

- Coupled reactions convert some, but not all, of the metabolic energy of glucose into ATP
- Under cellular conditions, approximately 50% of the energy of released from glycolysis converted to ATP chemical bond formation.
- Coupled reactions involving ATP hydrolysis are also used to drive the glycolytic pathway

The First Phase of Glycolysis

- Starting material: 1 X Glucose
- Ending material: 2 X Glyceraldehyde-3-phosphate (G-3-P)
- How do these phosphates come from?
- How many ATP should be used here?

In the first phase of glycolysis, five reactions convert a molecule of glucose to two molecules of glyceraldehyde-3-phosphate.



Rx 1. The first priming reaction

α-D-glucose + ATP^{4-}

Hexokinase/ Glucokinase

$\Delta G^{0'} = -16.7$ kJ/mol

α-D-glucose-6-phosphate^{2-} + ADP^{3-} + H^+

What if this reaction is carried out in cell?

[α-D-glucose] = 8.3 X 10^{-5} M

[ATP] = 1.85 X 10^{-3} M

[α-D-glucose-6-phosphate^{2-}] = 5.0 X 10^{-3} M

[ADP] = 1.4 X 10^{-4} M

Hexokinase

1st step in glycolysis; ΔG large, negative

- Hexokinase (and glucokinase) act to phosphorylate glucose and keep it in the cell
- K_m for glucose is 0.1 m*M*; cell has 4 m*M* glucose
- So hexokinase is normally active!
- Glucokinase ($K_m^{glucose}$ = 10 m*M*) only turns on when cell is rich in glucose (in liver)
- Hexokinase is regulated - allosterically inhibited by (product) glucose-6-P - but is not the most important site of regulation of glycolysis - Why?

Glucose is kept in the cell by phosphorylation to glucose-6-phosphate

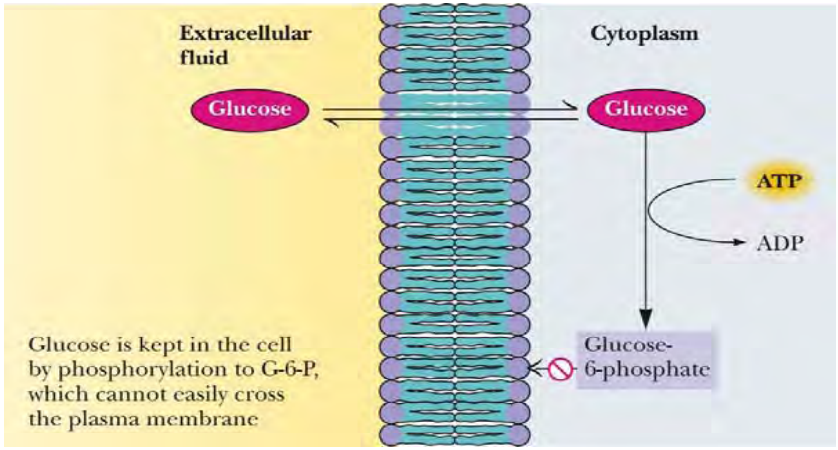


Figure 18.4 Glucose-6-P cannot cross the plasma membrane.

Steady-State Concentrations of Glycolytic Intermediates

These steady-state concentrations are used to obtain the cellular values of ΔG found in Table 18.1 and Figure 18.22.

TABLE 18.2 Steady-State Concentrations of Glycolytic Metabolites in Erythrocytes

Metabolite	mM
Glucose	5.0
Glucose-6-phosphate	0.083
Fructose-6-phosphate	0.014
Fructose-1,6-bisphosphate	0.031
Dihydroxyacetone phosphate	0.14
Glyceraldehyde-3-phosphate	0.019
1,3-Bisphosphoglycerate	0.001
2,3-Bisphosphoglycerate	4.0
3-Phosphoglycerate	0.12
2-Phosphoglycerate	0.030
Phosphoenolpyruvate	0.023
Pyruvate	0.051
Lactate	2.9
ATP	1.85
ADP	0.14
P_i	1.0

Glucose-6-P is common to several metabolic pathways

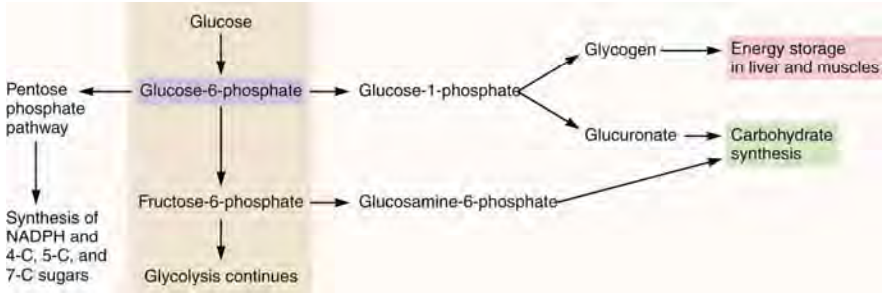


Figure 18.5 Glucose-6-phosphate is the branch point for several metabolic pathways.

The Structure of Hexokinase

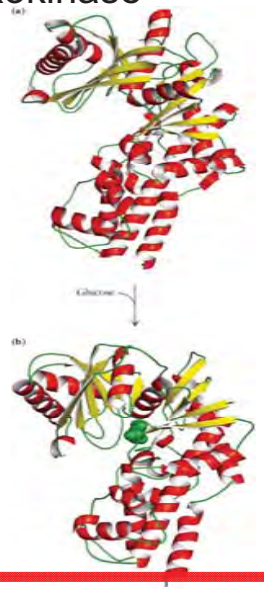


Figure 18.6 The (a) open and (b) closed states of yeast hexokinase. Binding of glucose (green) induces a conformation change that closes the active site, as predicted by Daniel Koshland. The induced fit model for enzymes is discussed on page 409 of the text.

Hexokinase is the paradigm of induced fit

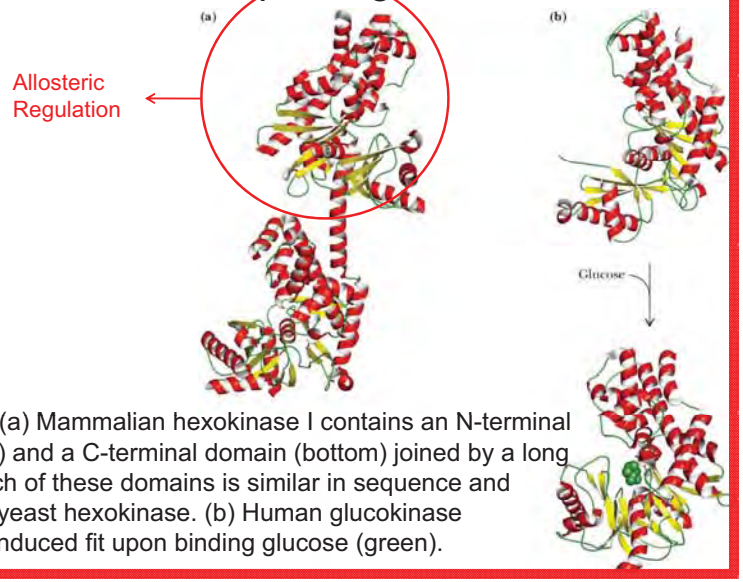


Figure 18.7 (a) Mammalian hexokinase I contains an N-terminal domain (top) and a C-terminal domain (bottom) joined by a long α-helix. Each of these domains is similar in sequence and structure to yeast hexokinase. (b) Human glucokinase undergoes induced fit upon binding glucose (green).

Rx 2: Phosphoglucoisomerase

Glucose-6-P to Fructose-6-P

- Why does this reaction occur??
  - next step (phosphorylation at C-1) would be tough for hemiacetal -OH, but easy for primary -OH
  - isomerization activates C-3 for cleavage in aldolase reaction
- Ene-diol intermediate in this reaction
- Equilibrium reaction!

A mechanism for phosphoglucoisomerase

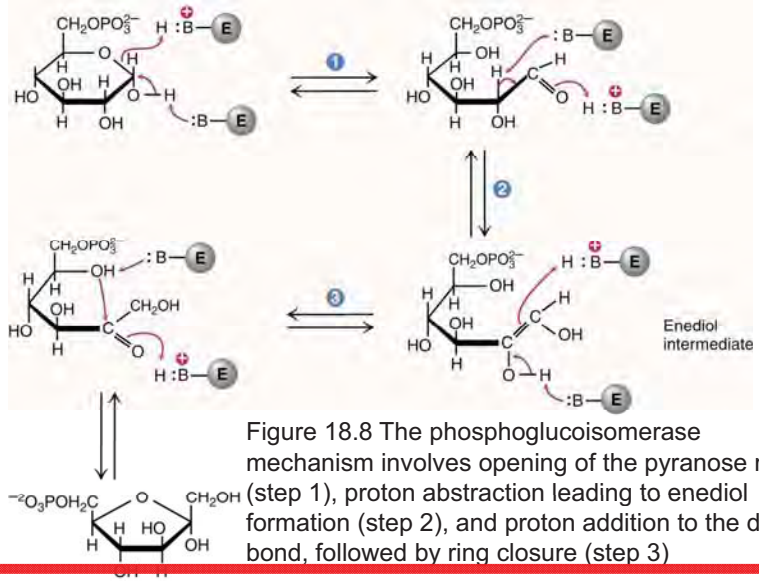


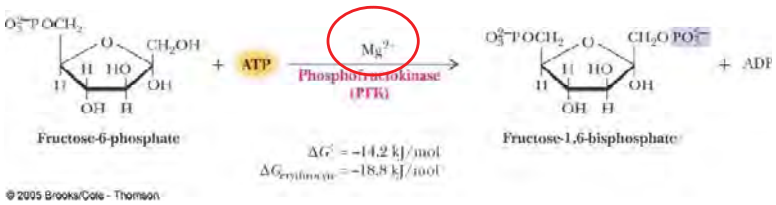
Figure 18.8 The phosphoglucoisomerase mechanism involves opening of the pyranose ring (step 1), proton abstraction leading to enediol formation (step 2), and proton addition to the double bond, followed by ring closure (step 3)

Rx 3: Phosphofructokinase

PFK is the committed step in glycolysis!

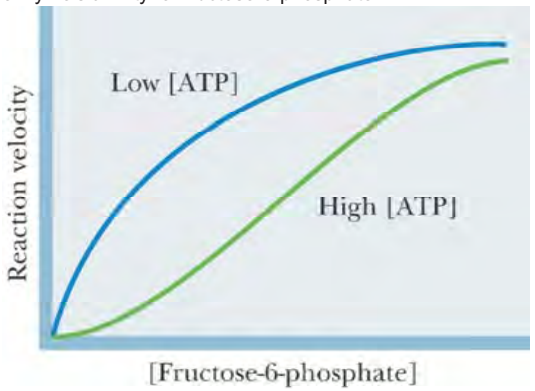
- The second priming reaction and regulation point of glycolysis
- Committed step and large, neg delta G - means PFK is highly regulated
- ATP inhibits, AMP reverses inhibition
- Citrate is also an allosteric inhibitor
- Fructose-2,6-bisphosphate is allosteric activator

Important in all priming reaction



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

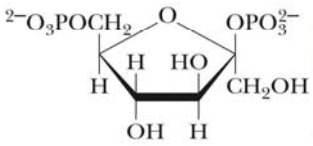
At high [ATP], phosphofructokinase (PFK) behaves cooperatively and the plot of enzyme activity versus [fructose-6-phosphate] is sigmoid. High [ATP] thus inhibits PFK, decreasing the enzyme's affinity for fructose-6-phosphate.



ATP level changes lower than 10%, but the effect is so significant! Why? Adenylate kinase!

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.

Fructose-2,6-BP
1. Increase F-1-P binding
2. Decease ATP inhibition
3. Inhibit F-1,6-BP phosphatase……



Fructose-2,6-bisphosphate

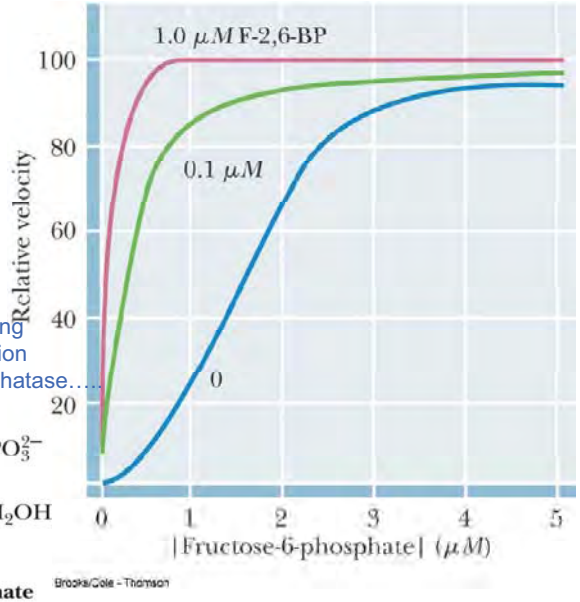
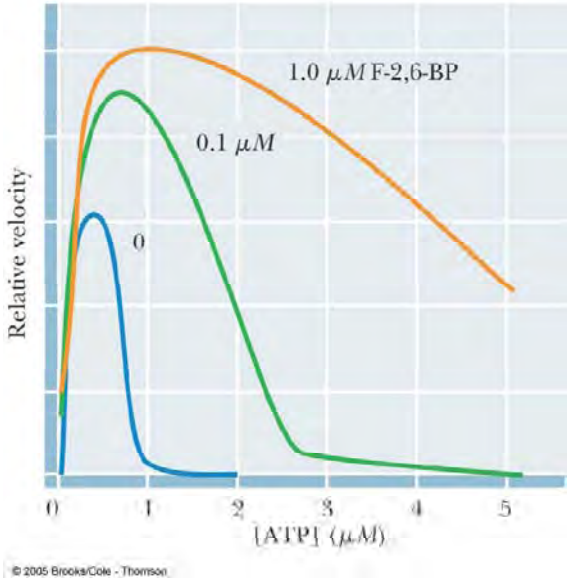


Figure 18.11
Fructose-2,6-bisphosphate decreases the inhibition of phosphofructokinase due to ATP.

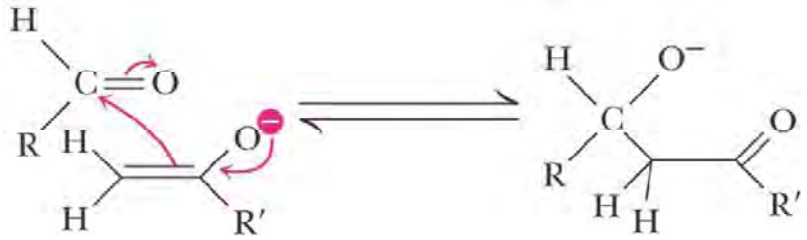


Rx 4: Aldolase

C_6 is cleaved to 2 C_3s (DHAP, Gly-3-P)

- Animal aldolases are Class I aldolases
- Class I aldolases form covalent Schiff base intermediate between substrate and active site lysine (inhibit by borohydride)
- Understand the evidence for Schiff base intermediate (box on page 590)

Aldol = aldehyde + alcohol



R′ = H (aldehyde)
R′ = alkyl, etc. (ketone)

The fructose-1,6-bisphosphate aldolase reaction.

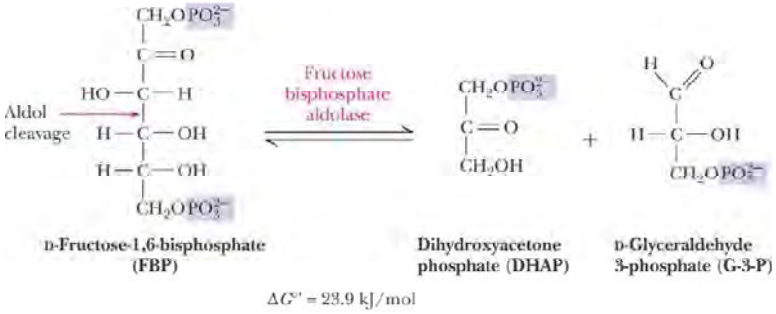
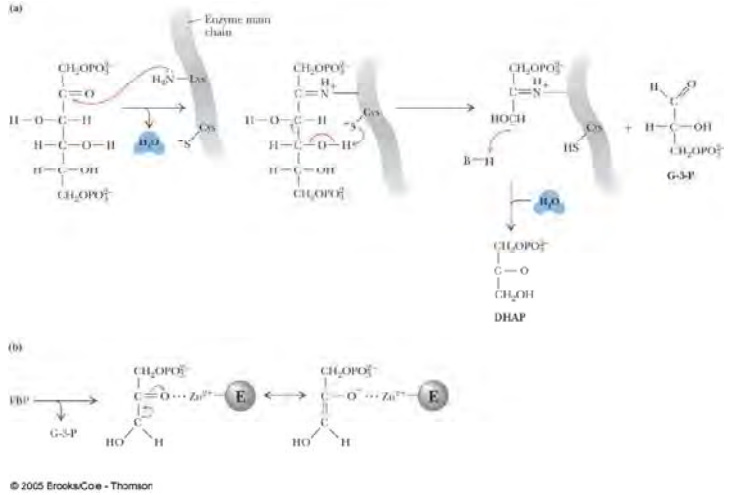


Figure 18.12
(a) A mechanism for the fructose-1,6-bisphosphate aldolase reaction. The Schiff base formed between the substrate carbonyl and an active-site lysine acts as an electron sink, increasing the acidity of the β-hydroxyl group and facilitating cleavage as shown. **(b)** In Class II aldolases, an active-site Zn^{2+} stabilizes the enolate intermediate, leading to polarization of the substrate carbonyl group.



A Class II aldolase mechanism

(b)

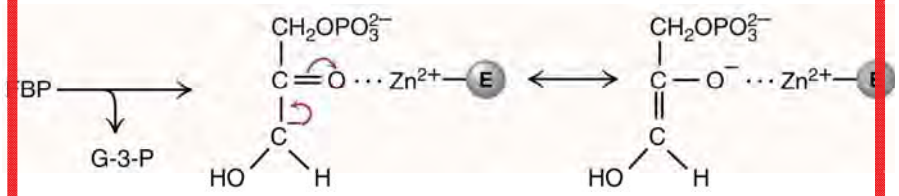


Figure 18.12 (b) In Class II aldolases, an active-site Zn^{2+} stabilizes the enolate intermediate, leading to polarization of the substrate carbonyl group.

Rx 5: Triose Phosphate Isomerase

DHAP is converted to G3-P

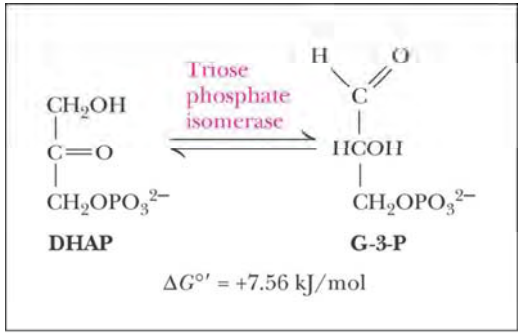
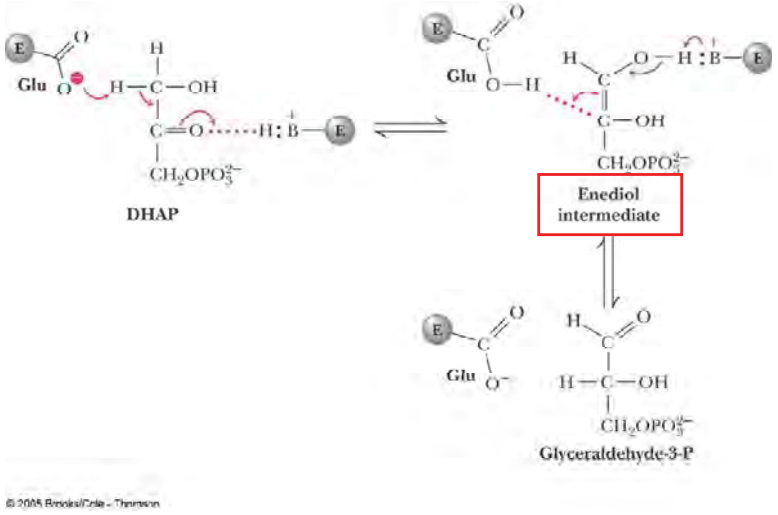


Figure 18.13
A reaction mechanism for triose phosphate isomerase.



Interconversion of triose phosphate by triose phosphate isomerase: C-1, C-2, C-3 of the starting glucose are chemically indistinguishable from C-4, C-5 and C-6 after the reaction.

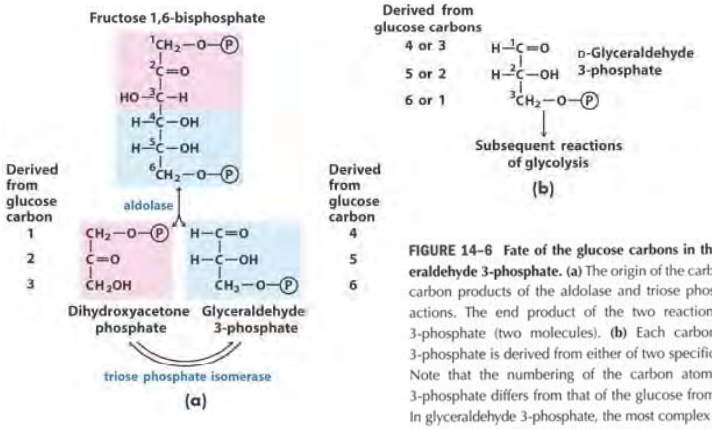


FIGURE 14–6 Fate of the glucose carbons in the formation of glyceraldehyde 3-phosphate. **(a)** The origin of the carbons in the two three-carbon products of the aldolase and triose phosphate isomerase reactions. The end product of the two reactions is glyceraldehyde 3-phosphate (two molecules). **(b)** Each carbon of glyceraldehyde 3-phosphate is derived from either of two specific carbons of glucose. Note that the numbering of the carbon atoms of glyceraldehyde 3-phosphate differs from that of the glucose from which it is derived. In glyceraldehyde 3-phosphate, the most complex functional group (the carbonyl) is specified as C-1. This numbering change is important for interpreting experiments with glucose in which a single carbon is labeled with a radioisotope. (See Problems 3 and 5 at the end of this chapter.)

End of Part 1

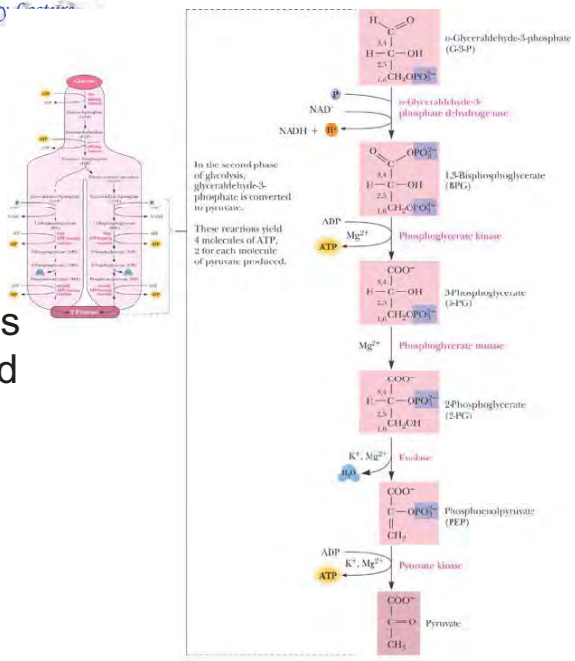
- Ask yourself…
  - What is the starting material of glycolysis and ending materials of first phase and second phase?
  - What are the 3 fates of pyruvate?
  - What are the 5 enzymes involved in the first phase of glycolysis?
  - Which enzymes are regulated enzymes?

18.4 – What Are the Chemical Principles and Features of the Second Phase of Glycolysis?

Metabolic energy produces 4 ATP

- Net ATP yield for glycolysis is two ATP
- Second phase involves two very high energy phosphate intermediates
  - 1,3 BPG
  - Phosphoenolpyruvate

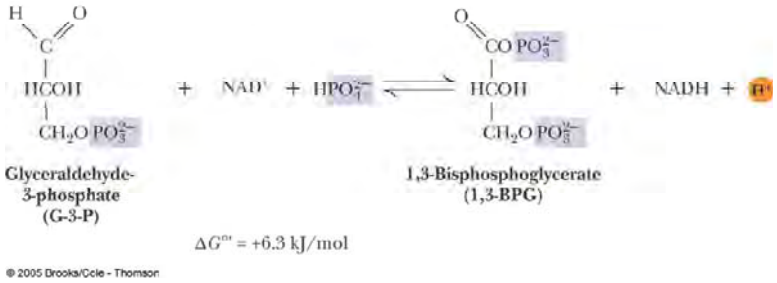
The second phase of glycolysis. Carbon atoms are numbered to show their original positions in glucose.



Rx 6: G-3-Dehydrogenase

G-3-P is oxidized to 1,3-BPG

- Energy yield from converting an aldehyde to a carboxylic acid is used to make 1,3-BPG and NADH
- Mechanism involves covalent catalysis and a nicotinamide coenzyme - know it



Nicotinic Acid and the Nicotinamide Coenzymes

aka pyridine nucleotides, vitamin B_3

- These coenzymes are two-electron carriers
- They transfer hydride anion ($H:^-$) to and from substrates
- Two important coenzymes in this class:
  - Nicotinamide adenine dinucleotide (NAD^+)
  - Nicotinamide adenine dinucleotide phosphate ($NADP^+$)

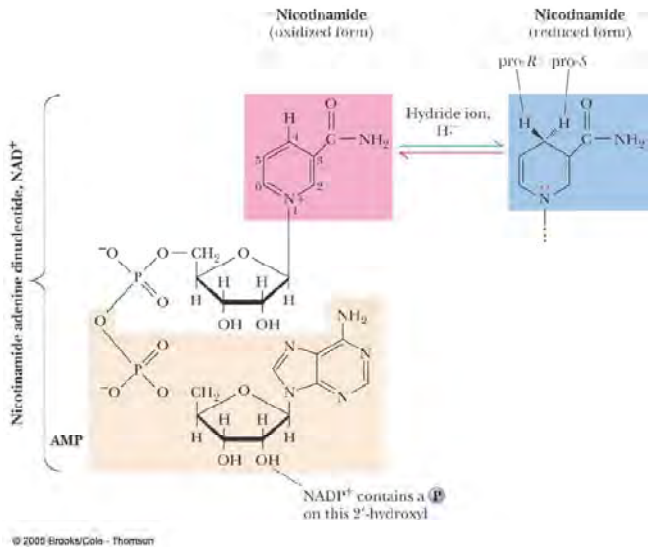
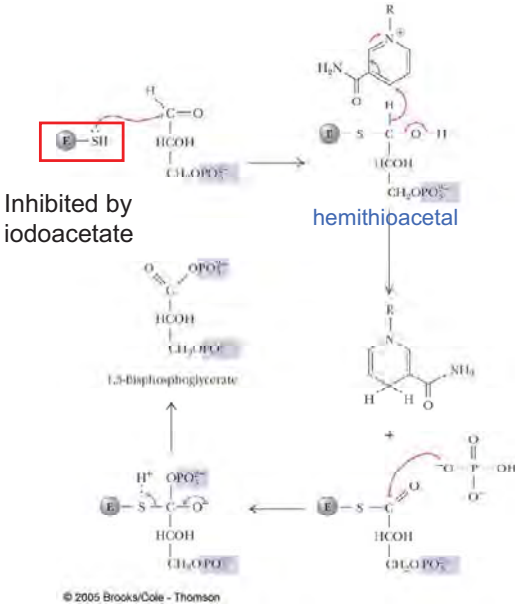
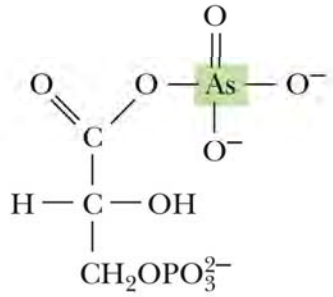


Figure 18.14
A mechanism for the glyceraldehyde-3-phosphate dehydrogenase reaction. Reaction of an enzyme sulfhydryl with the carbonyl carbon of glyceraldehyde-3-P forms a thiohemiacetal, which loses a hydride to NAD^+ to become a thioester. Phosphorolysis of this thioester releases 1,3-bisphosphoglycerate.



G3P-DH is the site of action of arsenate



1-Arseno-3-phosphoglycerate

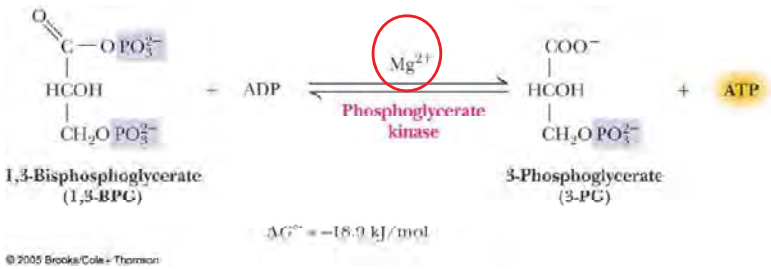
Arsenate is a substrate for the G3P-DH reaction, forming 1-arseno-3-phosphoglycerate. This product breaks down to 3-phosphoglycerate, essentially bypassing the phosphoglycerate kinase reaction. The result is that glycolysis in the presence of arsenate produces no net ATP.

Rx 7: Phosphoglycerate Kinase

ATP synthesis from a high-energy phosphate

- Pays off!
- This is referred to as "substrate-level phosphorylation" (the other is oxidative phosphorylation)

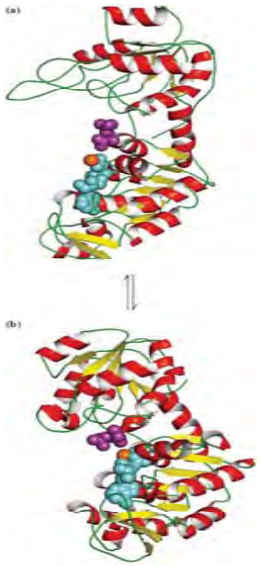
The phosphoglycerate kinase reaction.



Reaction 7 pulls reaction 6 forward!

Structure of Phosphoglycerate Kinase

The open (a) and closed (b) forms of phosphoglycerate kinase. ATP (cyan), 3-phosphoglycerate (purple), and Mg^{2+} (gold).



2,3-BPG is made by reactions that detour around the phosphoglycerate kinase rxn

- 2,3-bisphosphoglycerate is an important regulator of hemoglobin (see Chap15)
- 2,3-BPG is formed from 1,3-BPG by bisphosphoglycerate mutase
- 3-phosphoglycerate is then formed by 2,3-bisphosphoglycerate phosphatase
- Most cells contain only a trace of 2,3-BPG, but erythrocytes typically contain 4-5 mM 2,3-BPG

2,3-BPG is made by reactions that detour around the phosphoglycerate kinase rxn

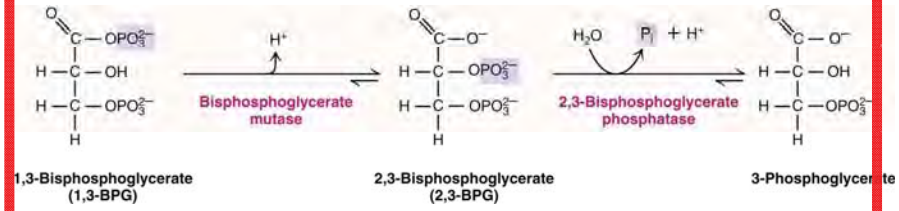


Figure 18.15 Formation and decomposition of 2,3-bisphosphglycerate.

2,3-BPG is made by reactions that detour around the phosphoglycerate kinase rxn

Figure 18.16 The mutase that forms 2,3-BPG from 1,3-BPG requires 3-phosphoglycerate. The reaction is actually an intermolecular phosphoryl transfer from C-1 of 1,3-BPG to C-2 of 3-phosphoglycerate.

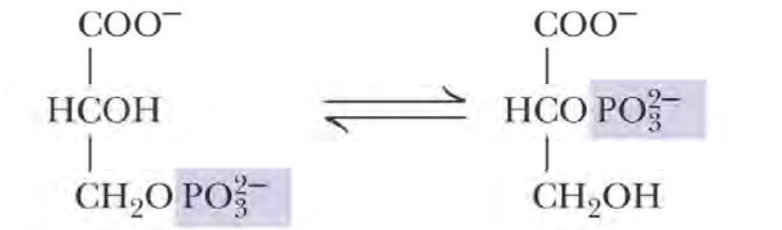
Rx 8: Phosphoglycerate Mutase

Phosphoryl group from C-3 to C-2

- Mutase: catalyze migration of a functional group within a molecule!
- Rationale for this enzyme - repositions the phosphate to make PEP
- Two types of phosphoglycerate mutase
  - Rabbit (need 2,3-bisphosphoglycerate)
  - Wheat (no cofactor)

Hanjia's Biochemistry Lecture

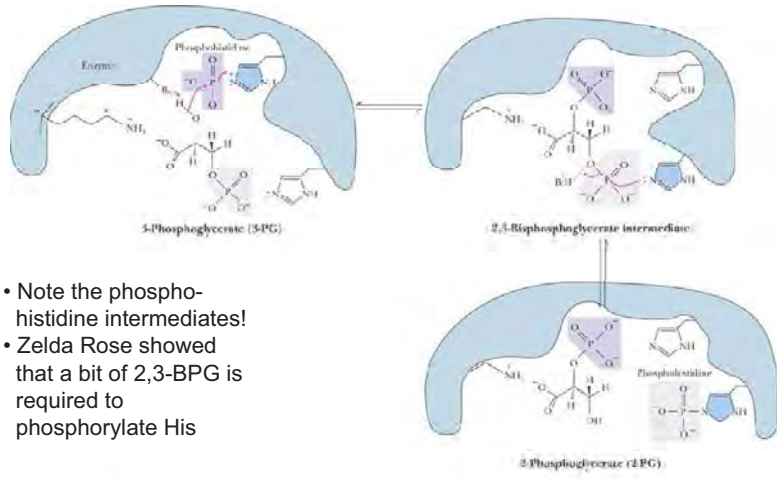
The phosphoglycerate mutase reaction.



3-Phosphoglycerate (3-PG) **2-Phosphoglycerate (2-PG)**

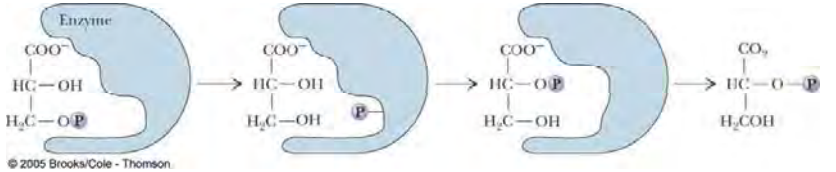
$\Delta G^{\circ\prime} = +4.4$ kJ/mol

A mechanism for the phosphoglycerate mutase reaction in rabbit muscle and in yeast. Zelda Rose of the Institute for Cancer Research in Philadelphia showed that the enzyme requires a small amount of 2,3-BPG to phosphorylate the histidine residue before the mechanism can proceed. Prior to her work, the role of the phosphohistidine in this mechanism was not understood.



- Note the phospho-histidine intermediates!
- Zelda Rose showed that a bit of 2,3-BPG is required to phosphorylate His

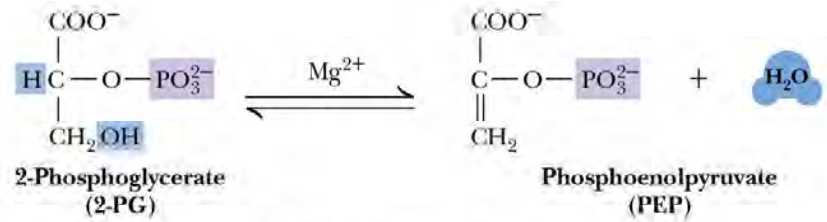
The phosphoglycerate mutase of wheat germ catalyzes an intramolecular phosphoryl transfer.

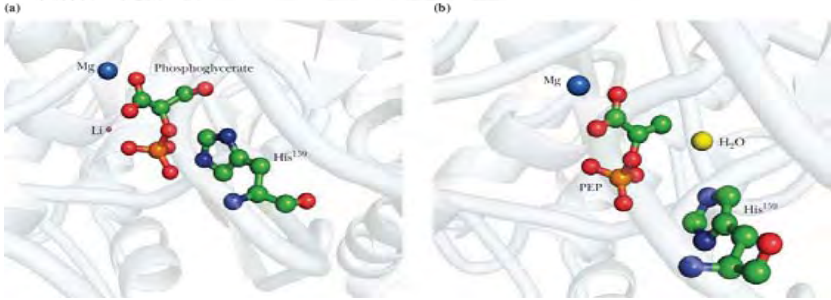


Rx 9: Enolase

2-P-G to PEP

- Overall ΔG is 1.8 kJ/mol
- How can such a reaction create a PEP?
- "Energy content" of 2-PG and PEP are similar
- Enolase just rearranges 2-PG to a form from which more energy can be released in hydrolysis

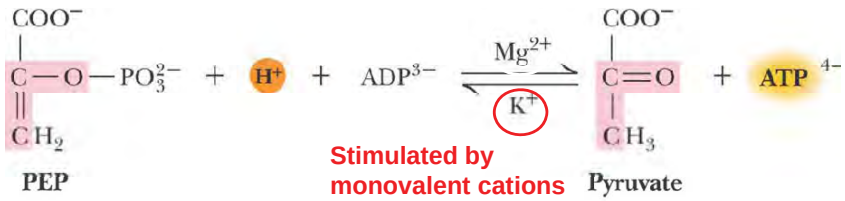




Rx 10: Pyruvate Kinase

PEP to Pyruvate makes ATP

- These two ATP (from one glucose) can be viewed as the "payoff" of glycolysis
- Large, negative ΔG - regulation!
- Allosterically activated by AMP, F-1,6-bisP
- Allosterically inhibited by ATP, acetyl-CoA and alanine



Stimulated by monovalent cations

$\Delta G^{\circ\prime} = -31.7$ kJ/mol

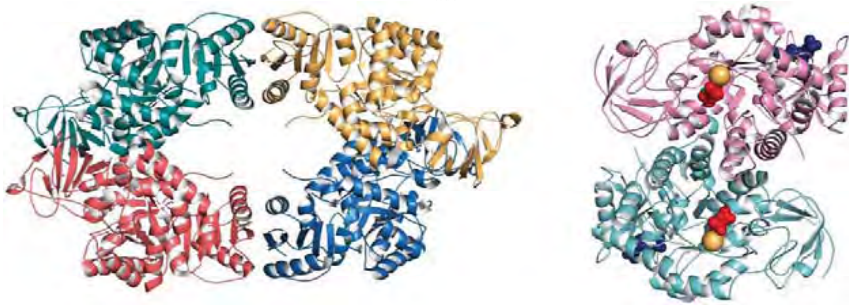
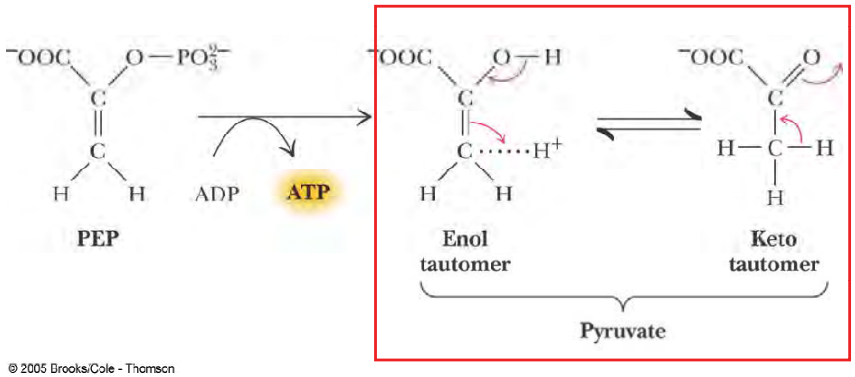
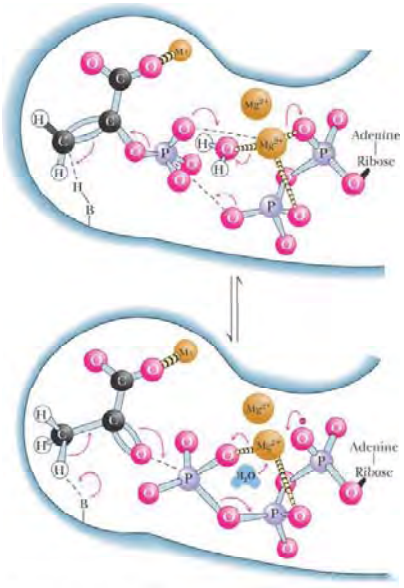


Figure 18.19
The conversion of phosphoenolpyruvate (PEP) to pyruvate may be viewed as involving two steps: phosphoryl transfer followed by an enol-keto tautomerization. The tautomerization is spontaneous ($\Delta G^{\circ\prime}$ = -35-40 kJ/mol) and accounts for much of the free energy change for PEP hydrolysis.



Contribute to negative free energy!

Figure 18.20
A mechanism for the pyruvate kinase reaction, based on NMR and EPR studies by Albert Mildvan and colleagues. Phosphoryl transfer from phosphoenolpyruvate (PEP) to ADP occurs in four steps: **(1)** a water on the Mg^{2+} ion coordinated to ADP is replaced by the phosphoryl group of PEP; **(2)** Mg^{2+} dissociates from the α-P of ADP; **(3)** the phosphoryl group is transferred; and **(4)** the enolate of pyruvate is protonated. (*Adapted from Mildvan*, A., 1979. *The role of metals in enzyme-catalyzed substitutions at each of the phosphorus atoms of ATP.* Advances in Enzymology **49:**103-126.)



Covalent modification of Pyruvate kinase

- Glucagon (hormone) stimulated phosphorylation of pyruvate kinase (by PKC)
- Two effects:
  1. More sensitive to ATP and alanine
  2. Higher Km for PEP

End of Part 2

- Ask yourself…
  - What are the 2 payoff reactions?
  - What are the 2 high energy intermediate?
  - How many ATP are produced here?
  - How many ways to regulate the activity of Pyruvate kinase?
  - What is the function of NAD+?

18.5 – What Are the Metabolic Fates of NADH and Pyruvate Produced in Glycolysis?

Aerobic or anaerobic??

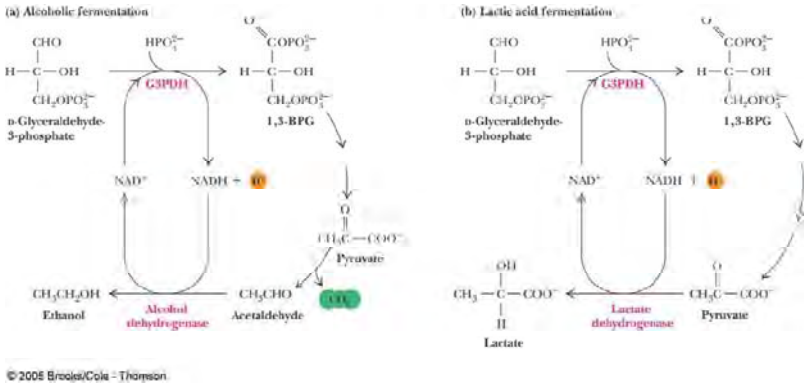
- NADH is energy - two possible fates:
  - If O_2 is available, NADH is re-oxidized in the electron transport pathway, making ATP in oxidative phosphorylation
  - In anaerobic conditions, NADH is re-oxidized by lactate dehydrogenase (LDH), providing additional NAD^+ for more glycolysis

The Fate of NADH and Pyr

Aerobic or anaerobic??

- Pyruvate is also energy - two possible fates:
  - aerobic: citric acid cycle
  - anaerobic: LDH makes lactate

Figure 18.21
(a) Pyruvate reduction to ethanol in yeast provides a means for regenerating NAD^+ consumed in the glyceraldehyde-3-P dehydrogenase reaction. **(b)** In oxygen-depleted muscle, NAD^+ is regenerated in the lactate dehydrogenase reaction.

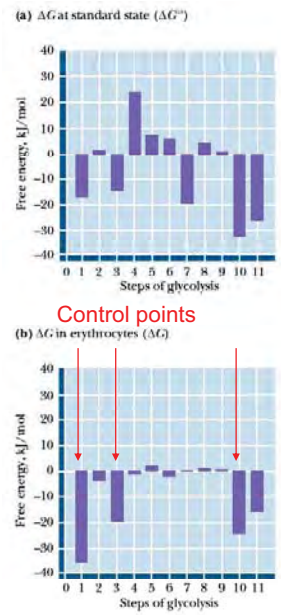


18.6 – How Do Cells Regulate Glycolysis?

The elegant evidence of regulation!

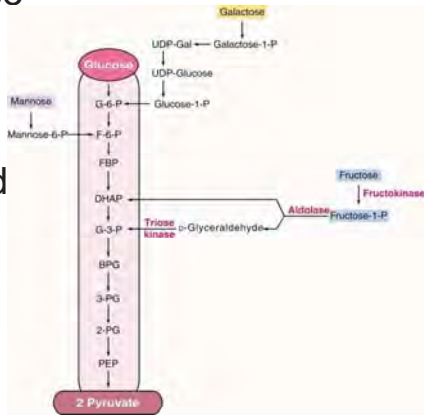
- Standard state ΔG values are scattered: + and -
- ΔG in cells is revealing:
  - Most values near zero
  - 3 of 10 Rxns have large, negative ΔG
- Large negative ΔG Rxns are sites of regulation!

Figure 18.22
A comparison of free energy changes for the reactions of glycolysis (step 1 = hexokinase) under **(a)** standard-state conditions and **(b)** actual intracellular conditions in erythrocytes. The values of $\Delta G^{\circ\prime}$ provide little insight into the actual free energy changes that occur in glycolysis. On the other hand, under intracellular conditions, seven of the glycolytic reactions operate near equilibrium (with ΔG near zero). The driving force for glycolysis lies in the hexokinase (1), phosphofructokinase (3), and pyruvate kinase (10) reactions. The lactate dehydrogenase (step 11) reaction also exhibits a large negative ΔG under cellular conditions.

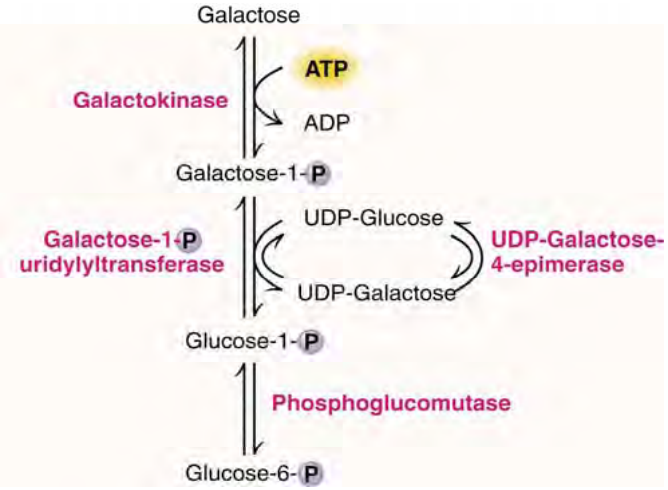


18.7 Are Substrates Other Than Glucose Used in Glycolysis?

- Sugars other than glucose can be glycolytic substrates
- Fructose, mannose and galactose can all be used
- Fructose and mannose are routed into glycolysis by fairly conventional means.
- Galactose is more interesting



The Leloir pathway



Galactose Enters Glycolysis Via the Leloir Pathway

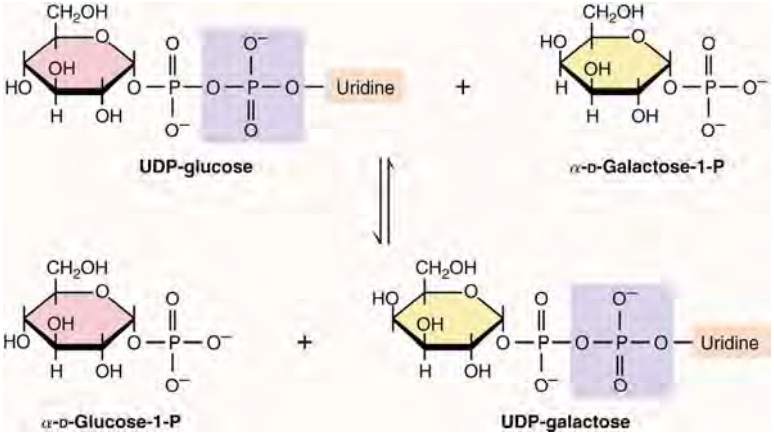
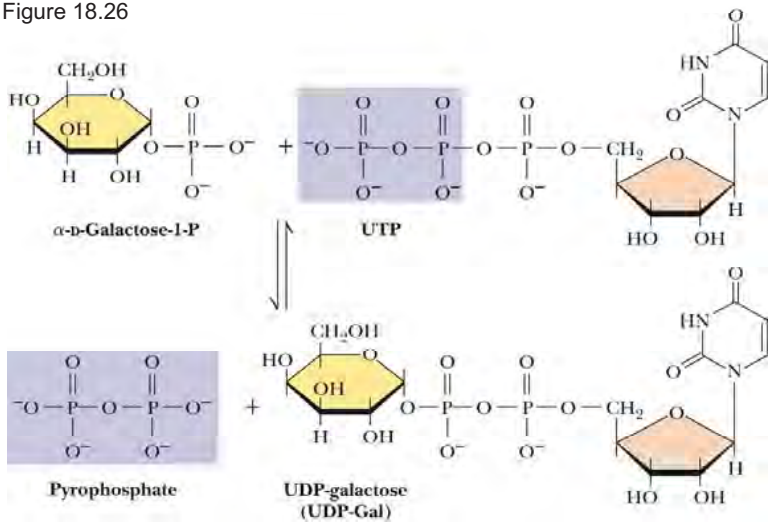


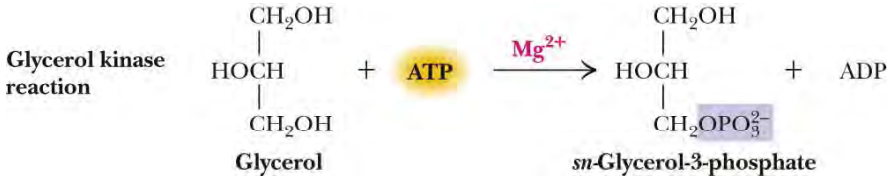
Figure 18.25 The galactose-1-phosphate uridylyltransferase reaction involves a "ping-pong" kinetic mechanism.

UDP-glucose pyrophosphorylase uses Gal-1-P, reducing galactose toxicity in adults

Figure 18.26

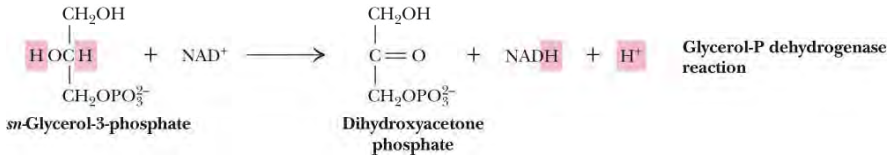


Glycerol Can Also Enter Glycolysis



Glycerol is produced in the decomposition of triacylglycerols. It can be converted to glycerol-3-P by glycerol kinase. Glycerol-3-P is then oxidized to dehydroxyacetone phosphate by the action of glycerol phosphate dehydrogenase.

Glycerol Can Also Enter Glycolysis

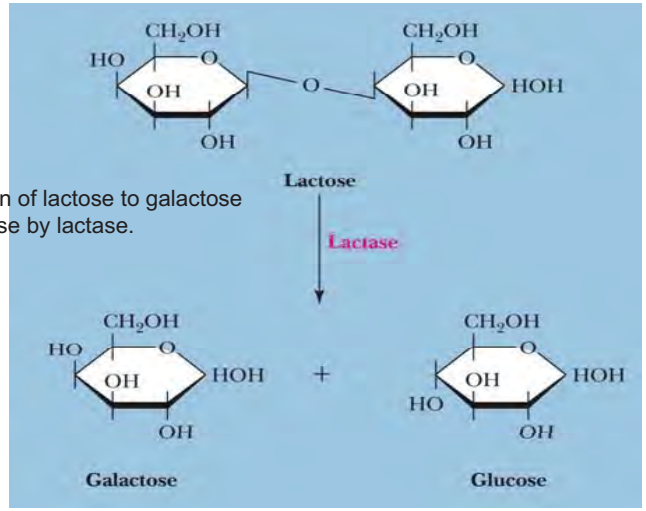


Glycerol is produced in the decomposition of triacylglycerols. It can be converted to glycerol-3-P by glycerol kinase. Glycerol-3-P is then oxidized to dehydroxyacetone phosphate by the action of glycerol phosphate dehydrogenase.

Lactose – From Mother's Milk to Yogurt – and Lactose Intolerance

- In placental mammals, lactose is synthesize only in the mammary gland, and then only during late pregnancy and lactation
- The synthesis is done by **lactose synthase**, a dimeric complex of galactosyl transferase and α-lactalbumin
- Lactose breakdown in the intestines by **lactase** provides newborns with essential galactose
- Some humans are **lactose intolerant**, due to a particularly low level of lactase
- Lactic acid fermentation by certain bacteria is the basis for the production of **yogurt**

Lactose – From Mother's Milk to Yogurt – and Lactose Intolerance



Breakdown of lactose to galactose and glucose by lactase.

Many Humans are Lactose Intolerant Due to a Low Level of Lactase

Percentage of Population with Lactase Persistence

Country	Lactase Persistence (%)
Sweden	99
Denmark	97
United Kingdom (Scotland)	95
Germany	88
Australia	82
United States (Iowa)	81
Spain	72
France	58
India	36
Japan	10
China (Singapore)	0

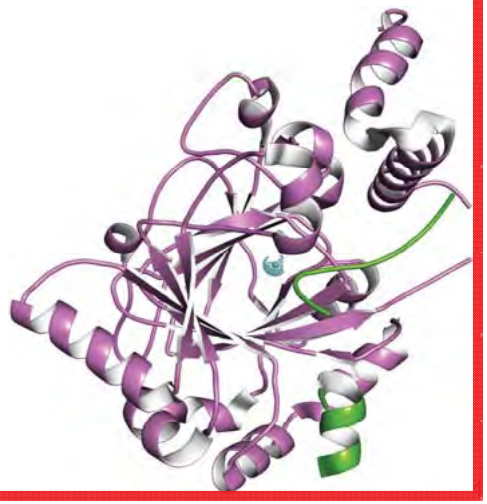
18.8 How Do Cells Respond to Hypoxic Stress?

- Glycolysis is an **anaerobic** pathway
- The tricarboxylic acid cycle is **aerobic**
- When oxygen is abundant, cells prefer to combine these pathways in **aerobic metabolism**
- When oxygen is limiting, cells adapt to carry out more glycolysis
- **Hypoxia** causes changes in gene expression that increases levels of glycolytic enzymes
- A trigger for this is a DNA binding protein called **hypoxia inducible factor (HIF)**
- HIF is regulated at high oxygen levels by **hydroxylase factor-inhibiting HIF (FIH-1)**

18.8 How Do Cells Respond to Hypoxic Stress?

Figure 18.27 FIH (green) bound to HIF.

Hydroxylation of HIF-1α Asn^{803} by FIH in the presence of oxygen inhibits the transcription activity of HIF-1α.



18.8 How Do Cells Respond to Hypoxic Stress?

- HIF consists of two subunits: a ubiquitous HIF-1β subunit and a hypoxia-responsive HIF-1α subunit
- In response to hypoxia, inactivation of the prolyl hydroxylases allows
  - HIF-1α stabilization
  - Dimerization with HIF-1β
  - Binding of the dimer to the hypoxia responsive element (HRE) of HIF target genes
  - Activation of transcription of these genes
- VHL is the "von Hippel Lindau subunit of the ubiquitin E3 ligase that targets proteins for proteasome degradation

18.8 How Do Cells Respond to Hypoxic Stress?

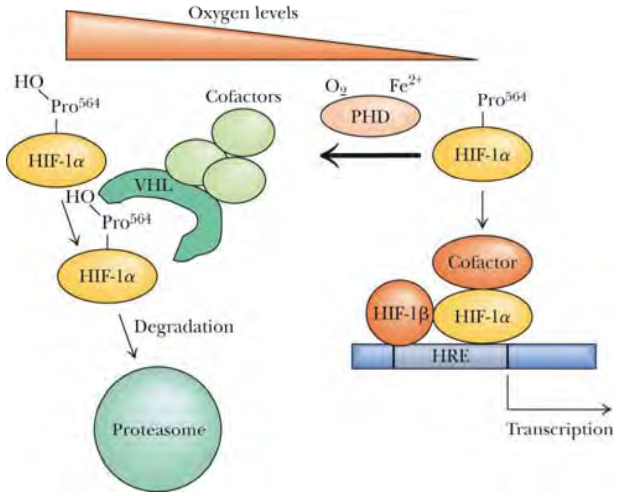


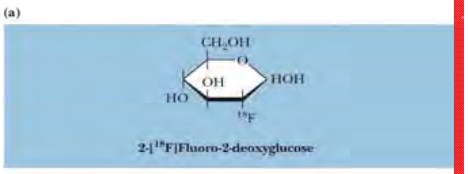
Figure 18.28

Detection of Tumor by Glycolysis

- Tumors show very high rates of glycolysis, as shown by Otto Warburg early in the 20th century
- This observation is the basis of tumor detection by **positron emission tomography (PET)**
- Metabolites labeled with ^{18}F can be taken up by human cells (in the brain, for example)
- Decay of ^{18}F results in positron emission
- Positron-electron collisions produce gamma rays
- Detection with gamma ray cameras provides 3D models of tumor extent and location
- 2-[^{18}F]fluoro-2-deoxy-glucose, used for this purpose, is a substrate for hexokinase

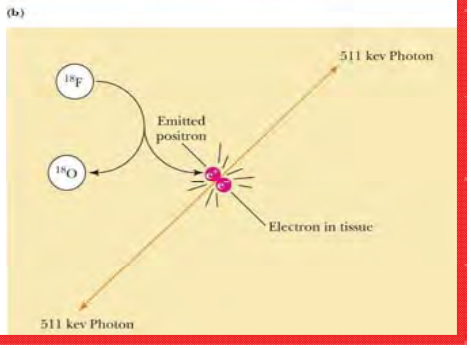
Tumor Diagnosis Using Positron Emission Tomography (PET)

2-[^{18}F]fluoro-2-deoxy-glucose is a substrate for hexokinase

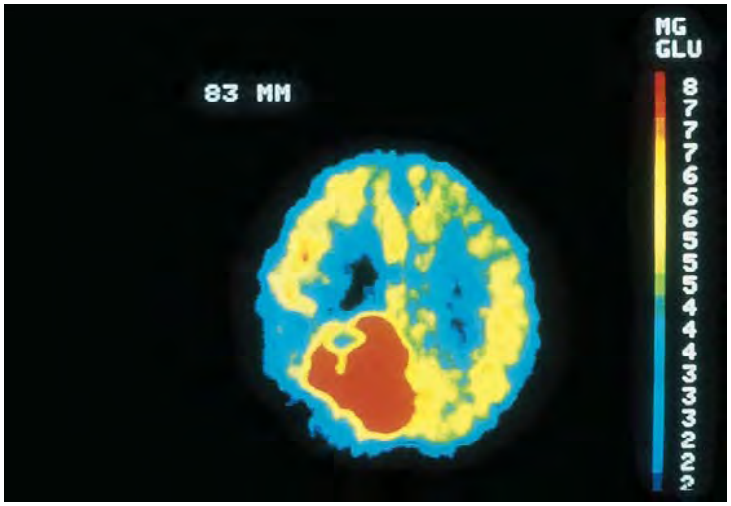


Decay of ^{18}F results in positron emission.

Positron-electron collisions produce gamma rays



Detection with gamma ray cameras provides 3D models of tumor extent and location



Hanjia's Biochemistry Lecture

End of Part 3

- Ask your self…
  - What are the fates of NADH and Pyruvate?
  - How do other sugar enter glycolysis pathway?
  - What is the relationship between hypoxia and glycolysis?

End of the class

- You should have learned
  - The ten steps of glycolysis
  - The regulation enzymes in glycolysis
  - The energy yield in glycolysis
  - The possible fates of the products of glycolysis
  - The relationship of hypoxia and glycolysis