Chapter 11 - Glycolysis

- For centuries, bakeries and breweries have exploited the conversion of glucose to ethanol and CO_2 by glycolysis in yeast

11.1 Glycolysis Is a Ubiquitous Pathway

Converts: **1 glucose ⟹ 2 pyruvate**

- Pyruvate can be further metabolized to:
  (1) Lactate or ethanol (anaerobic)
  (2) Acetyl CoA (aerobic)
- Acetyl CoA is further oxidized to CO_2 and H_2O via the citric acid cycle
- Much more ATP is generated from the citric acid cycle than from glycolysis

Fig 11.1

- Catabolism of glucose via glycolysis and the citric acid cycle

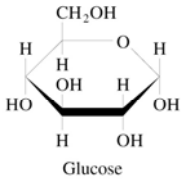

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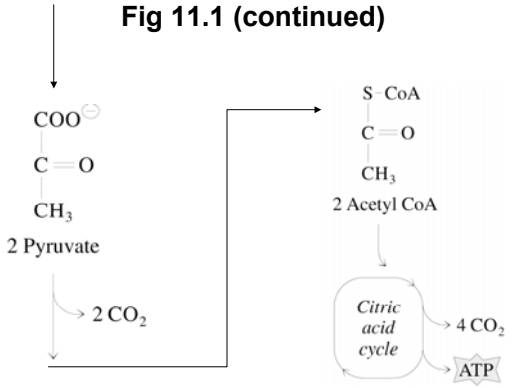
Fig 11.1 (continued)



Net reaction of glycolysis

- Two molecules of ATP are produced
- Two molecules of NAD^+ are reduced to NADH

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{NAD}^+ + 2\ \text{P}_i \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O}$$

Table 11.1

TABLE 11.1 The enzymatic reactions of glycolysis

Reaction	Enzyme
1. Glucose + ATP ⟶ Glucose6-phosphate + ADP + $H^{\oplus}$	Hexokinase,glucokinase
2. Glucose6-phosphate ⟺ Fructose 6-phosphate	Glucose-6-phosphateisomerase
3. Fructose6-phosphate + ATP ⟶ Fructose1,6-*bi*sphosphate + ADP + $H^{\oplus}$	Phosphofructokinase-1
4. Fructose1,6-*bi*sphosphate ⟺ Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate	Aldolase
5. Dihydroxyacetone phosphate ⟺ Glyceraldehyde 3-phosphate	Triose phosphateisomerase
6. Glyceraldehyde 3-phosphate + $NAD^{\oplus}$ + $_iP$ ⟺ 1,3-*Bis*phosphoglycerate + NADH + $H^{\oplus}$	Glyceraldehyde 3-phosphatedehydrogenase
7. 1,3-*Bi*sphosphoglycerate + ADP ⟺ 3-Phosphoglycerate + ATP	Phosphoglycerate kinase
8. 3-Phosphoglycerate ⟺ 2-Phosphoglycerate	Phosphoglyceratemutase
9. 2-Phosphoglycerate ⟺ Phosphoenolpyruvate + H_2O	Enolase
10. Phosphoenolpyruvate + ADP + $H^{\oplus}$ ⟶ Pyruvate + ATP	Pyruv

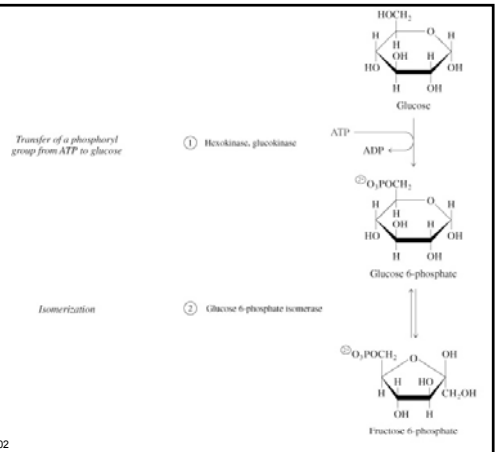
Glycolysis (10 reactions) can be divided into two stages

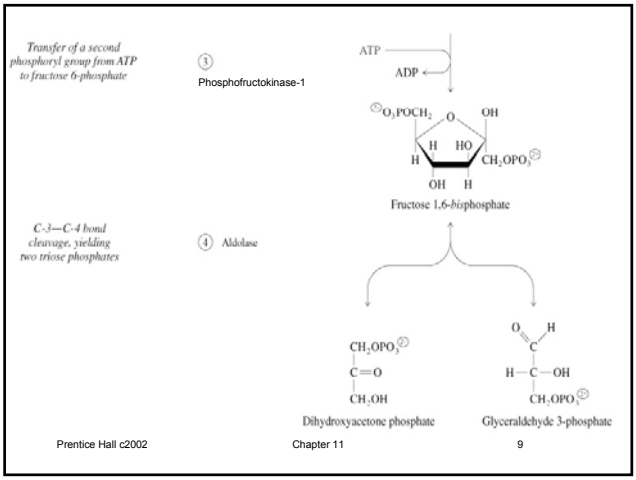
- Hexose stage: **2 ATP** are consumed per glucose
- Triose stage: **4 ATP** are produced per glucose

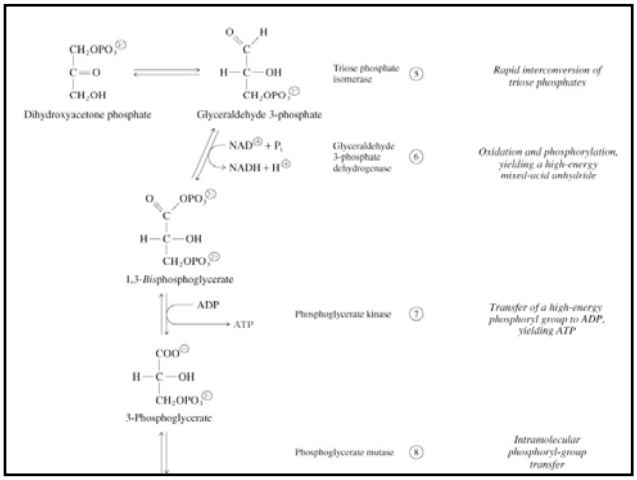
Net: **2 ATP** produced per glucose

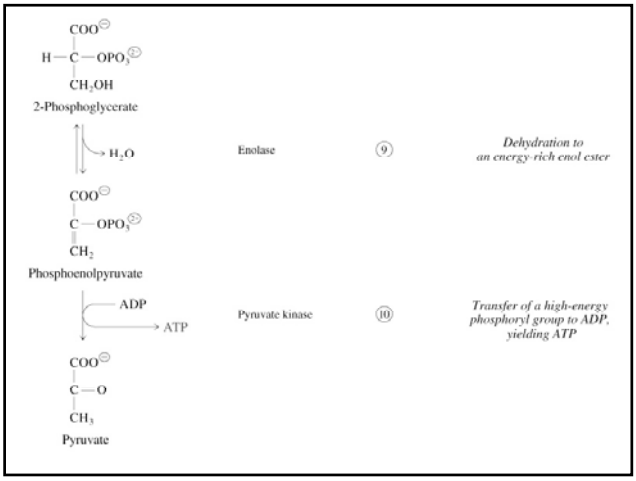
Fig 11.2

- Glycolysis (next 4 slides)









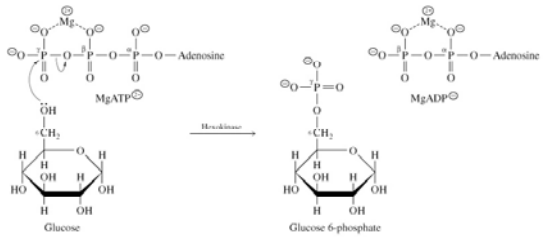
11.2 Glycolysis Has 10 Enzyme-Catalyzed Steps

- Each chemical reaction prepares a substrate for the next step in the process
- A hexose is cleaved to two trioses
- Interconversion of the trioses allows both to be further metabolized via glycolytic enzymes
- ATP is both consumed and produced in glycolysis

1. Hexokinase

- Transfers the γ-phosphoryl of ATP to **glucose** C-6 oxygen to generate **glucose 6-phosphate** (**G6P**)
- Mechanism: attack of C-6 hydroxyl oxygen of glucose on the γ-phosphorous of $MgATP^{2-}$ displacing $MgADP^{-}$
- Four kinases in glycolysis: steps 1,3,7, and 10
- All four kinases require Mg^{2+} and have a similar mechanism

Fig 11.3 Hexokinase reaction



Properties of hexokinases

- Broad substrate specificity - hexokinases can phosphorylate glucose, mannose and fructose
- Yeast hexokinase undergoes an induced-fit conformational change when glucose binds
- Conformational change helps prevent hydrolysis of ATP to ADP and P_i (Fig 6.13)

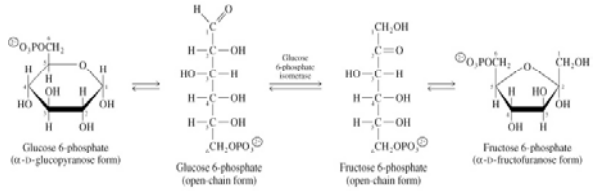
Isozymes of hexokinase

- **Isozymes** - multiple forms of hexokinase occur in mammalian tissues and yeast
- Hexokinases I, II, III are active at normal glucose concentrations (K_m values ~10^{-6} to 10^{-4}M)
- Hexokinase IV (**Glucokinase**, K_m ~10^{-2}M) is active at higher glucose levels, allows the liver to respond to large increases in blood glucose

2. Glucose 6-Phosphate Isomerase

- Converts **glucose 6-phosphate** (**G6P**) (an aldose) to **fructose 6-phosphate** (**F6P**) (a ketose)
- Enzyme preferentially binds the α-anomer of G6P (converts to open chain form in the active site)
- Enzyme is highly stereospecific for G6P and F6P
- Isomerase reaction is near-equilibrium in cells

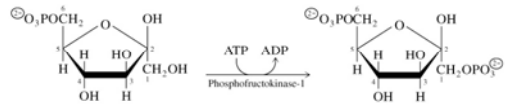
Fig 11.4 Conversion of G6P to F6P



3. Phosphofructokinase-1 (PFK-1)

- Catalyzes transfer of a phosphoryl group from ATP to the C-1 hydroxyl group of **F6P** to form **fructose 1,6-*bis*phosphate** (**F1,6BP**)
- PFK-1 is metabolically irreversible and a critical regulatory point for glycolysis in most cells (PFK-1 is the first committed step of glycolysis)
- A second phosphofructokinase (PFK-2) synthesizes **fructose 2,6-*bis*phosphate** (**F2,6BP**)

PFK-1 Reaction

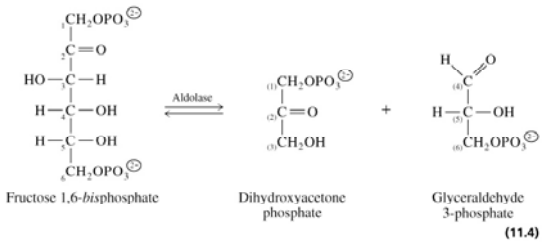


(11.3)

4. Aldolase

- Aldolase cleaves the hexose F1,6BP into two triose phosphates: **glyceraldehyde 3-phosphate** (**GAP**) and **dihydroxyacetone phosphate** (**DHAP**)
- Reaction is near-equilibrium, not a control point
- Mechanism is common for cleaving C-C bonds in biological systems (and C-C bond formation in the reverse direction)

Aldolase Reaction



(11.4)

Fig 11.5 Mechanism of aldolases

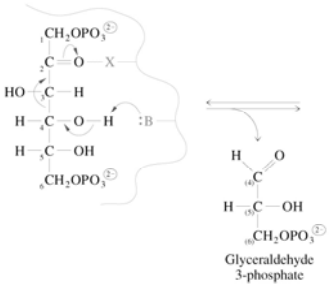


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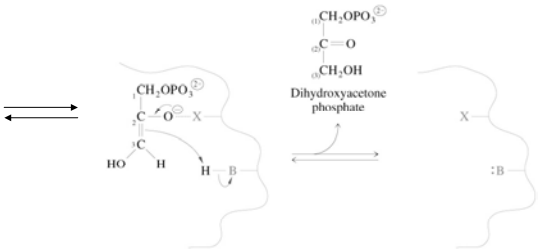
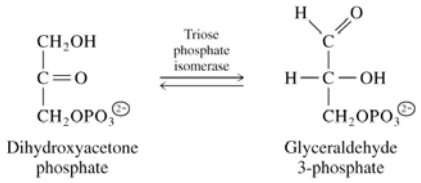
Fig 11.5 (continued)



5. Triose Phosphate Isomerase (TPI)

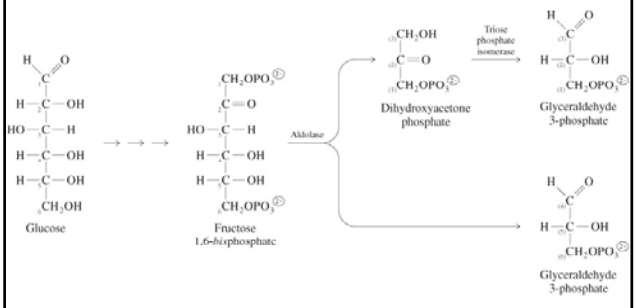
- Conversion of **DHAP** into **glyceraldehyde 3-phosphate** (**GAP**)
- Reaction is very fast (diffusion controlled), and only the D-isomer of GAP is formed
- Radioisotopic tracer studies show:
  One GAP molecule: C1,2,3 from Glucose C4,5,6
  Second GAP: C1,2,3 from Glucose C3,2,1

Reaction of Triose phosphate isomerase



(11.5)

Fig 11.6 Fate of carbon atoms from hexose stage to triose stage



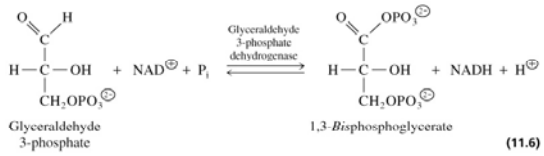
6. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)

- Conversion of **GAP** to **1,3-*bis*phosphoglycerate** (**1,3BPG**)
- Molecule of NAD^+ is reduced to NADH
- Oxidation of the aldehyde group of GAP proceeds with large negative free-energy change

Conservation of oxidative energy

- Energy from oxidation of GAP aldehyde is conserved in acid-anhydride linkage of 1,3BPG
- Next step of glycolysis uses the high-energy phosphate of 1,3BPG to form ATP from ADP
- Mechanism of GAPDH shows how an energy-rich compound forms in an oxidation reaction

Reaction of GAPDH: GAP converted to 1,3BPG



(11.6)

Fig 11.7

- Mechanism of GAPDH (3 slides)

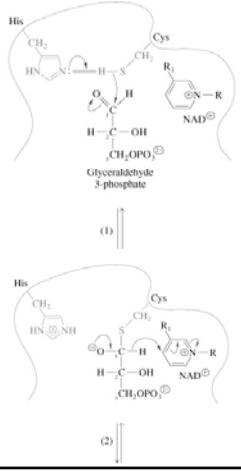


Fig 11.7 (continued)

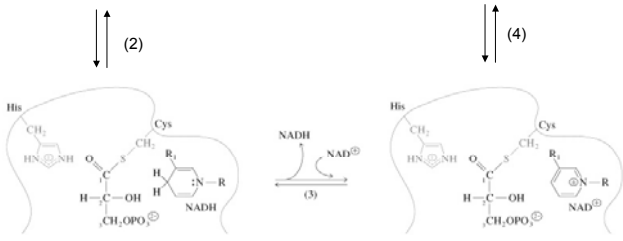
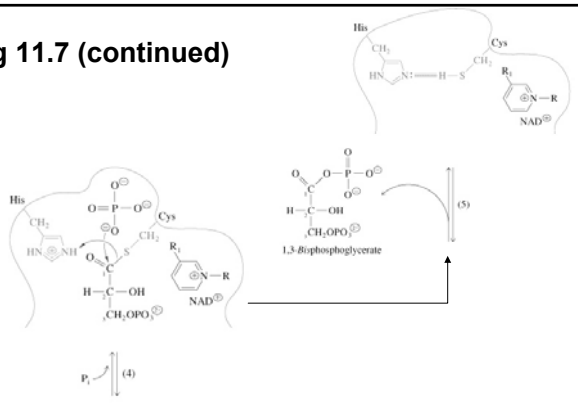
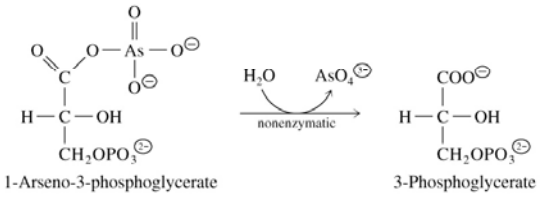


Fig 11.7 (continued)



Box 11.2 Arsenate (AsO_4^{3-}) poisoning

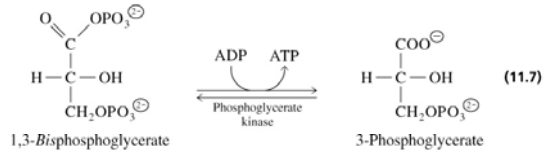
- Arsenate can replace P_i as a substrate for G3PDH
- Arseno analog which forms is unstable



7. Phosphoglycerate Kinase (PGK)

- Transfer of phosphoryl group from the energy-rich mixed anhydride 1,3BPG to ADP yields **ATP** and **3-phosphoglycerate** (**3PG**)
- **Substrate-level phosphorylation** - Steps 6 and 7 couple oxidation of an aldehyde to a carboxylic acid with the phosphorylation of ADP to ATP

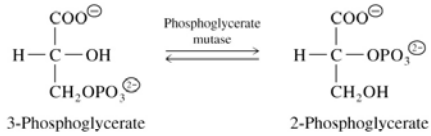
Phosphoglycerate kinase reaction



8. Phosphoglycerate Mutase

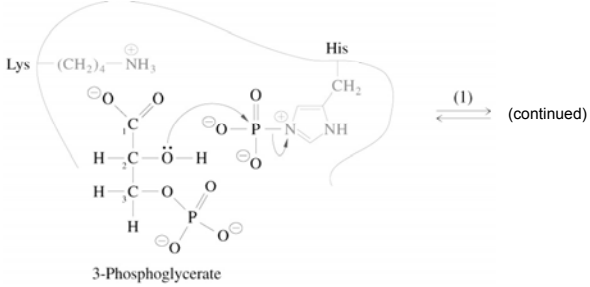
- Catalyzes transfer of a phosphoryl group from one part of a substrate molecule to another
- Reaction occurs without input of ATP energy
- Mechanism requires 2 phosphoryl-group transfer steps
- Enzymes from animal muscle and yeast have a different mechanism than does plant enzyme

Phosphoglycerate mutase reaction



3-Phosphoglycerate ⇌ 2-Phosphoglycerate (11.8)

Fig 11.8 Phosphoglycerate mutase mechanism: animals and yeast



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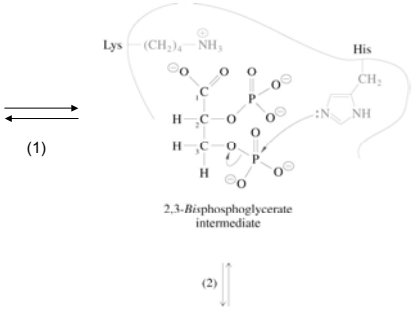
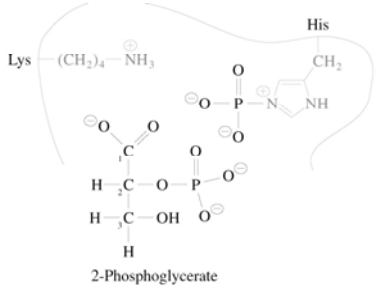


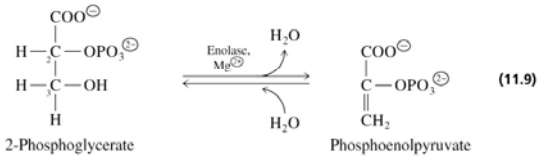
Fig 11.8 (continued)



9. Enolase: 2PG to PEP

- **3-Phosphoglycerate** (**3PG**) is dehydrated to **phosphoenolpyruvate** (**PEP**)
- Elimination of water from C-2 and C-3 yields the enol-phosphate PEP
- PEP has a very high phosphoryl group transfer potential because it exists in its unstable enol form

Enolase reaction



(11.9)

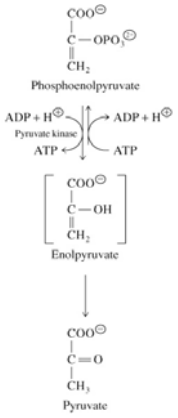
10. Pyruvate Kinase (PK)

PEP + ADP ⟶ Pyruvate + ATP

- Catalyzes a substrate-level phosphorylation
- Metabolically irreversible reaction
- Regulation both by allosteric modulators and by covalent modification
- Pyruvate kinase gene can be regulated by various hormones and nutrients

Fig 11.9

- Pyruvate kinase reaction

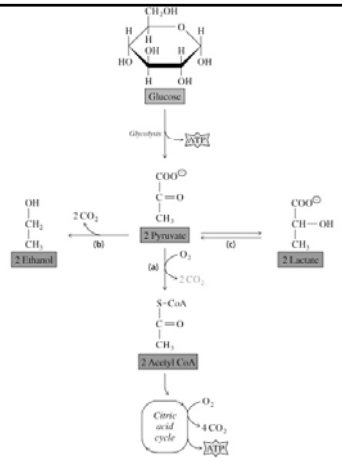


11.3 The Fate of Pyruvate

1. Aerobic conditions: oxidized to **acetyl CoA** which enters the citric acid cycle for further oxidation
2. Anaerobic conditions (microorganisms): conversion to **ethanol**
3. Anaerobic conditions (muscles, red blood cells): conversion to **lactate**

Fig 11.10

- Three major fates of pyruvate



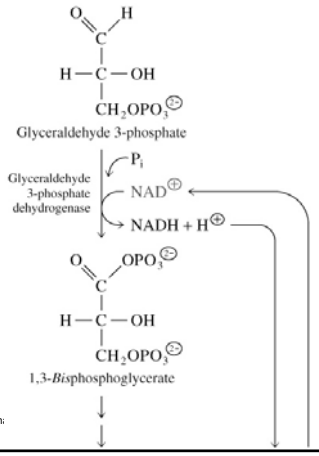
A. Metabolism of Pyruvate to Ethanol (yeast - anaerobic)

- Two reactions required:
  - (1) Pyruvate carboxylase
  - (2) Alcohol dehydrogenase

Pyruvate $\xrightarrow{(1)}$ **Acetaldehyde** $\xrightarrow{(2)}$ **Ethanol**

Fig 11.11

- Anaerobic conversion of pyruvate to ethanol (yeast)



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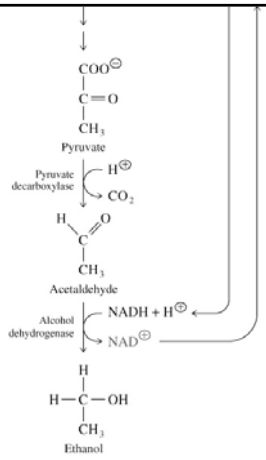


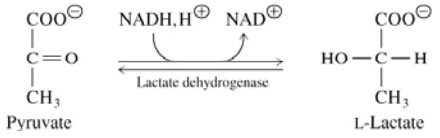
B. Reduction of Pyruvate to Lactate (muscles - anaerobic)

- Muscles lack pyruvate dehydrogenase and cannot produce ethanol from pyruvate
- Muscle lactate dehydrogenase converts pyruvate to lactate
- This reaction regenerates NAD^+ for use by GAPDH in glycolysis

Recycling of lactate

- Lactate formed in skeletal muscles during exercise is transported to the liver
- Liver lactate dehydrogenase can reconvert lactate to pyruvate
- Lactic acidosis can result from insufficient oxygen (an increase in lactic acid and decrease in blood pH)

Reduction of pyruvate to lactate



(11.11)

Overall reactions for glucose degradation to lactate

- Two ATP per molecule glucose consumed
- No oxygen is required

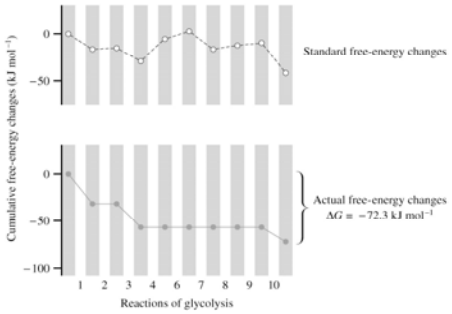
Glucose + 2 P_i^{2-} + 2 ADP^{3-} ⟹

2 $Lactate^-$ + 2 ATP^{4-} + 2 H_2O

11.4 Free-Energy Changes in Glycolysis

- Actual free-energy changes (ΔG) <u>large</u> only for:
  - #1 (hexokinase)
  - #3 (phosphofructokinase)
  - #10 (pyruvate kinase)
- These steps are <u>metabolically</u> <u>irreversible</u>, and these enzymes are <u>regulated</u>
- ΔG for all other steps are <u>close</u> <u>to</u> <u>zero</u> (they are near-equilibrium in cells)

Fig 11.12 Cumulative standard and actual free energy changes for the reactions of glycolysis



11.5 Regulation of Glycolysis

1. **When ATP is <u>needed</u>, glycolysis is <u>activated</u>**
- AMP and fructose 2,6-*bis*phosphate (F2,6BP) relieve the inhibition of PFK-1 by ATP
2. **When ATP levels are <u>sufficient</u>, glycolysis activity <u>decreases</u>**
- PFK-1 is inhibited by ATP and citrate
- Hexokinase inhibited by excess glucose 6-phosphate

Fig 11.13 Metabolic regulation of glycolysis

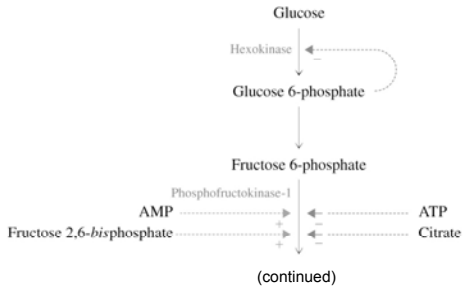
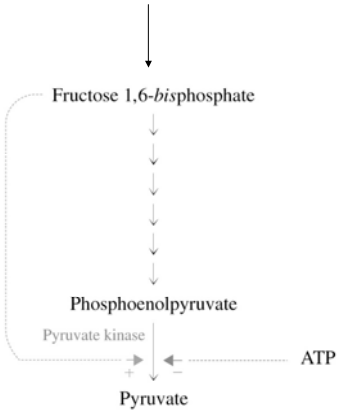


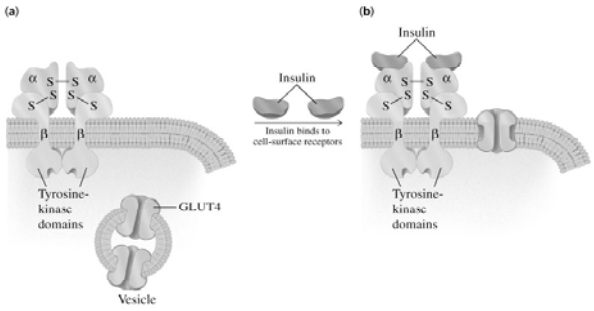
Fig 11.13 (continued)



A. Regulation of Hexose Transporters

- Glucose enters mammalian cells by passive transport down a concentration gradient from blood to cells
- GLUT is a family of six passive <u>hexose</u> <u>transporters</u>
- Glucose <u>uptake</u> into skeletal and heart muscle and adipocytes by GLUT 4 is <u>stimulated</u> <u>by</u> <u>insulin</u>
- Other GLUT transporters mediate glucose transport in and out of cells in the absence of insulin

Fig 11.14 Regulation of glucose transport by insulin

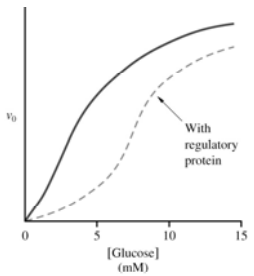


B. Regulation of Hexokinase

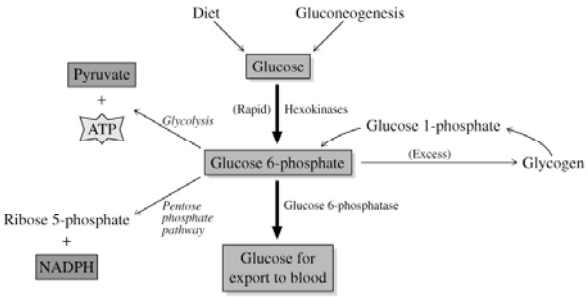
- Hexokinase reaction is metabolically irreversible
- G6P (product) levels increase when glycolysis is inhibited at sites further along in the pathway
- G6P inhibits hexokinase isozymes I, II and III
- Glucokinase forms G6P in the liver (for glycogen synthesis) when glucose is abundant (activity is modulated by fructose phosphates and a regulatory protein)

Fig 11.15 Effects of a regulatory protein on glucokinase kinetics

- Addition of a regulatory protein raises apparent K_m for glucose (inhibits glucokinase)



Box 11.3 Glucose 6-Phosphate Has a Pivotal Metabolic Role in Liver

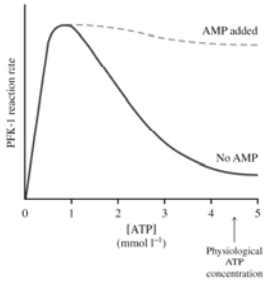


C. Regulation of Phosphofructokinase-1

- ATP is a substrate and an allosteric inhibitor of PFK-1
- AMP allosterically activates PFK-1 by relieving the ATP inhibition (ADP is also an activator in mammalian systems)
- Changes in AMP and ADP concentrations can control the flux through PFK-1
- Elevated levels of citrate (indicate ample substrates for citric acid cycle) also inhibit PFK-1

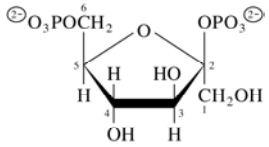
Fig 11.16 Regulation of PFK-1 by ATP and AMP

- AMP relieves ATP inhibition of PFK-1

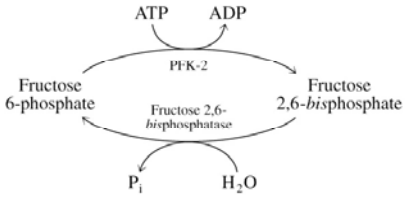


Regulation of PFK-1 by Fructose 2,6-*bis*phosphate (F2,6BP)

- F2,6BP is formed from F6P by the enzyme phosphofructokinase-2 (PFK-2)
- **Fig 11.17** β-D-Fructose 2,6-*bis*phosphate



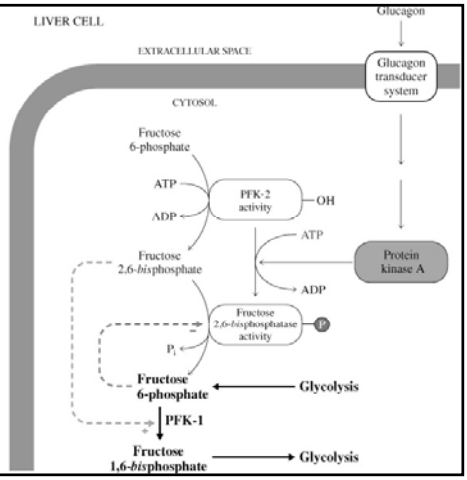
Formation and hydrolysis of F2,6BP



(11.15)

Fig. 11.18

- Effect of glucagon on liver glycolysis



D. Regulation of Pyruvate Kinase (PK)

- Four PK isozymes exist in mammalian tissues
- PK is allosterically activated by F1,6BP, inhibited by ATP
- Glucagon stimulates protein kinase A which phosphorylates PK converting it to a less active form (liver and intestinal cells)

Fig 11.19 Initial velocity curves of pyruvate kinase

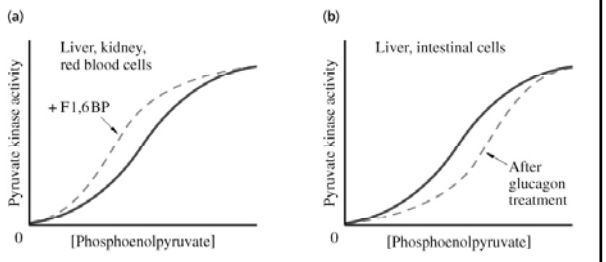
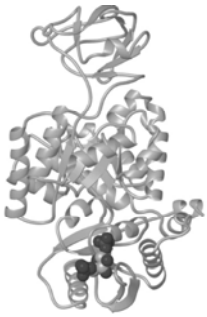


Fig 11.20 Pyruvate kinase with F1,6BP

- Activator F1,6BP (red)
- Active site is in large central domain



E. The Pasteur Effect

- Under anaerobic conditions the conversion of glucose to pyruvate is much higher than under aerobic conditions (yeast cells produce more ethanol and muscle cells accumulate lactate)
- The **Pasteur Effect** is the slowing of glycolysis in the presence of oxygen
- More ATP is produced under aerobic conditions than under anaerobic conditions, therefore less glucose is consumed aerobically

11.6 Other Sugars Can Enter Glycolysis

- Glucose is the main metabolic fuel in most organisms
- Other sugars convert to glycolytic intermediates
- Fructose and sucrose (contains fructose) are major sweeteners in many foods and beverages
- Galactose from milk lactose (a disaccharide)
- Mannose from dietary polysaccharides, glycoproteins

A. Fructose Is Converted to Glyceraldehyde 3-Phosphate

Fig 11.21

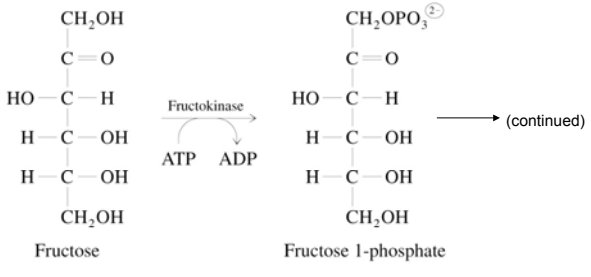
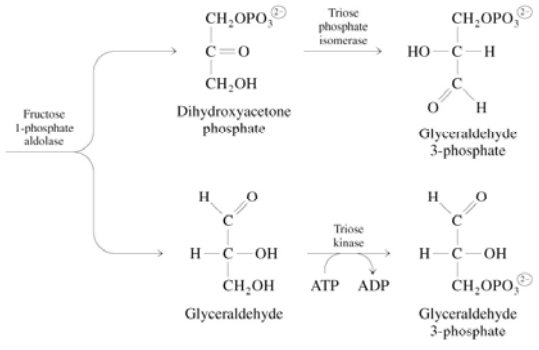


Fig 11.21 (continued)



B. Galactose is Converted to Glucose 1-Phosphate

Fig 11.22 (continued next slide)

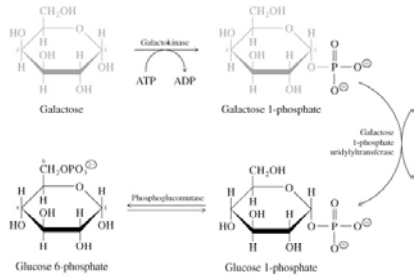
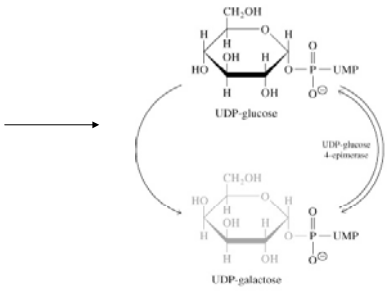
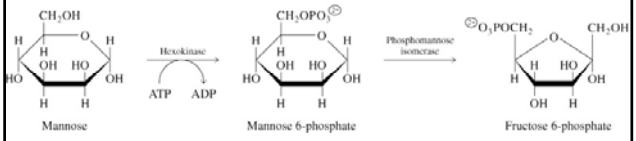


Fig 11.22 (continued)



C. Mannose is Converted to Fructose 6-Phosphate



11.7 Formation of 2,3-*Bis*phosphoglycerate in Red Blood Cells

- 2,3-*Bis*phosphoglycerate (2,3BPG) allosterically regulates hemoglobin oxygenation (red blood cells)
- Erythrocytes contain *bis*phosphoglycerate mutase which forms 2,3BPG from 1,3BPG
- In red blood cells about 20% of the glycolytic flux is diverted for the production of the important oxygen regulator 2,3BPG

Fig 11.24

- Formation of 2,3BPG in red blood cells