

GLYCOLYSIS

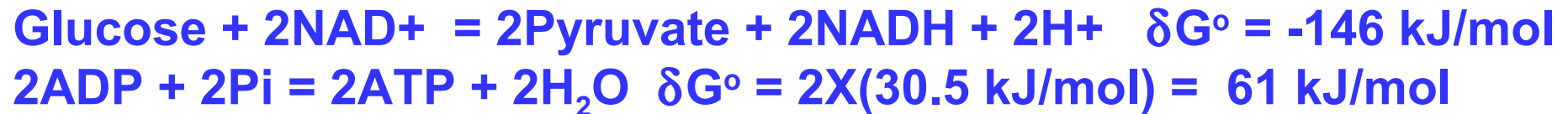
(Embden – Meyerhof Pathway)

Glycolysis

Glykys = Sweet, Lysis = splitting

During this process one molecule of glucose (6 carbon molecule) is degraded into two molecules of pyruvate (three carbon molecule).

Free energy released in this process is stored as 2 molecules of ATP, and 2 molecules of NADH.



$$\delta G^\circ (\text{overall}) = -146 + 61 = -85 \text{ kJ/mol}$$

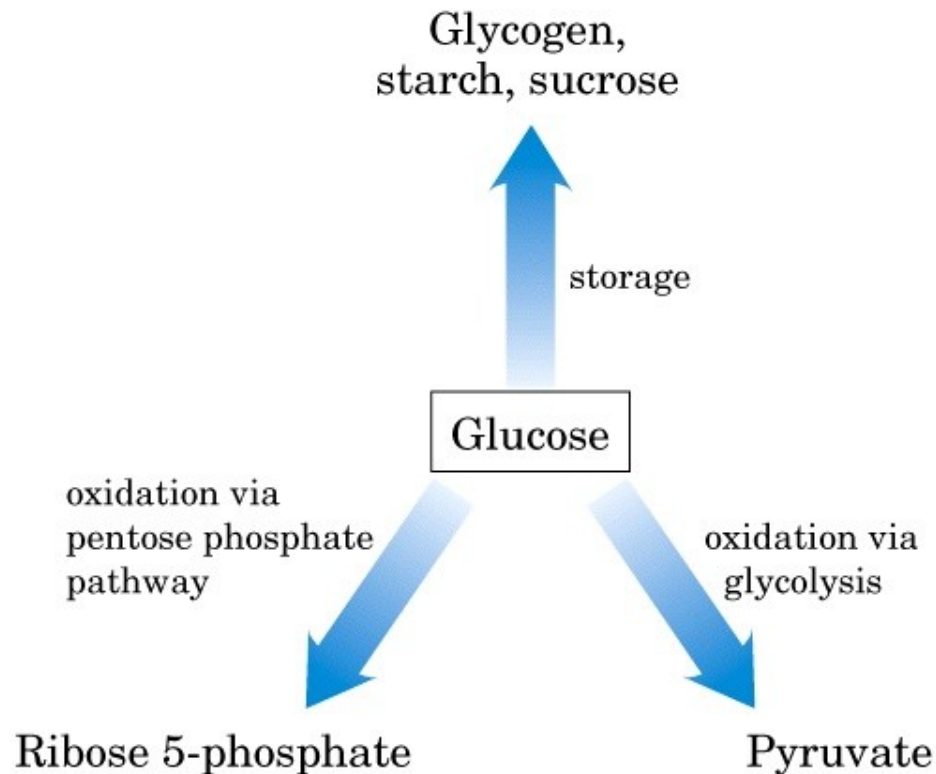
In standard condition glycolysis is an exergonic reaction which tends to be irreversible because of negative δG° .

- **It is also called as Embden-Meyerhof Pathway (EMP)**
- **it is defined as the sequence of reactions converting glucose or glycogen to pyruvate or lactate with production of ATP.**
- **Enzymes takes place in cytosomal fraction of the cell.**
- **major pathway in tissues lacking mitochondria like erythrocytes, cornea, lens etc.**
- **it is essential for brain which is dependent in glucose for energy.**
- **under anaerobic condition = glu + 2ADP + 2iP
-----→ 2 Lactate + 2ATP**

Fate of glucose in living systems



5.2% of total free energy that can be released by glucose is released in glycolysis.





Louis Pasteur (1822-1895)

Historical Perspective

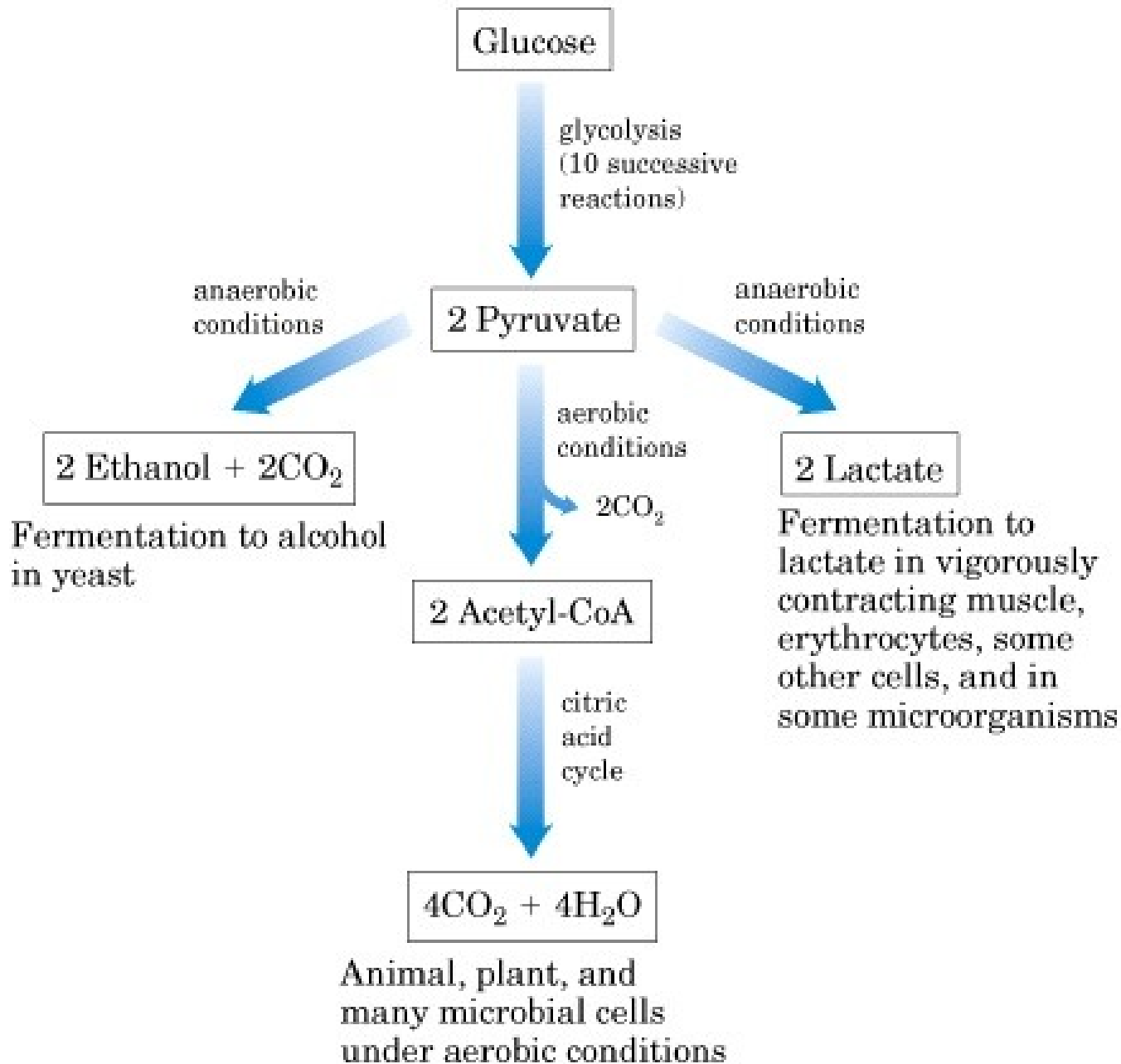
Glycolysis was the very first biochemistry or oldest biochemistry studied. It is the first metabolic pathway discovered.

Louis Pasteur 1854-1864: Fermentation is caused by microorganism. Pasteur's effect: Aerobic growth requires less glucose than anaerobic condition.

Buchner; 1897: Reactions of glycolysis can be carried out in cell-free yeast extract.

Harden and Young 1905: 1: inorganic phosphate is required for fermentation. 2: yeast extract could be separated in small molecular weight essential coenzymes or what they called Co-zymase and bigger molecules called enzymes or zymase.

1940: with the efforts of many workers, complete pathways for glycolysis was established.



There are 10 enzyme-catalyzed reactions in glycolysis.

There are two stages

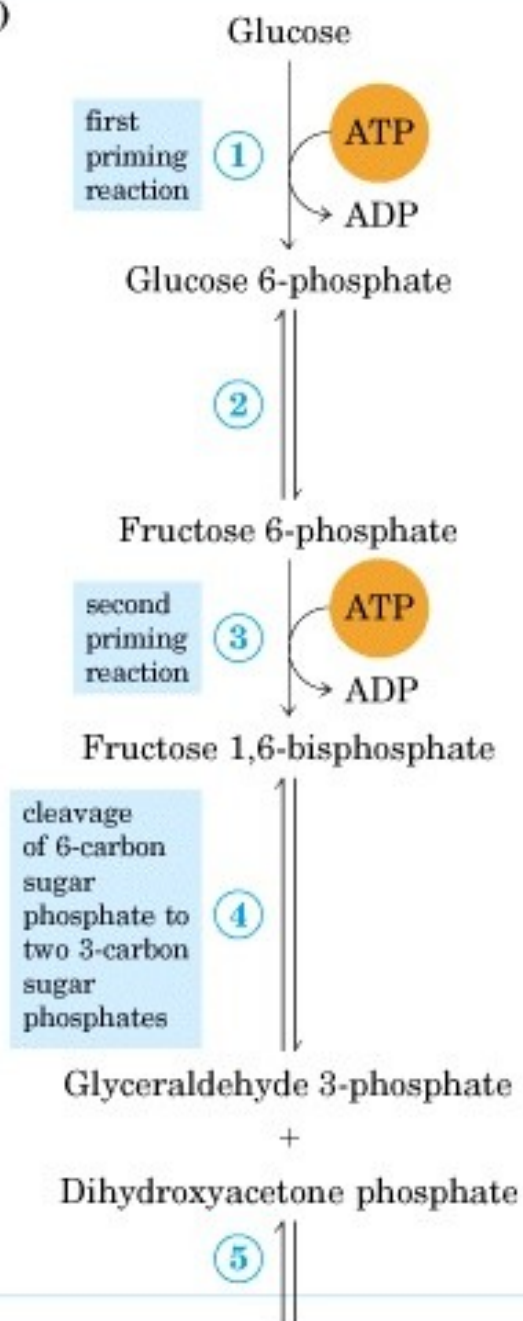
Stage 1: (Reactions 1-5) A preparatory stage in which glucose is phosphorylated, converted to fructose which is again phosphorylated and cleaved into two molecules of glyceraldehyde-3-phosphate. In this phase there is an investment of two molecules of ATP.

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:

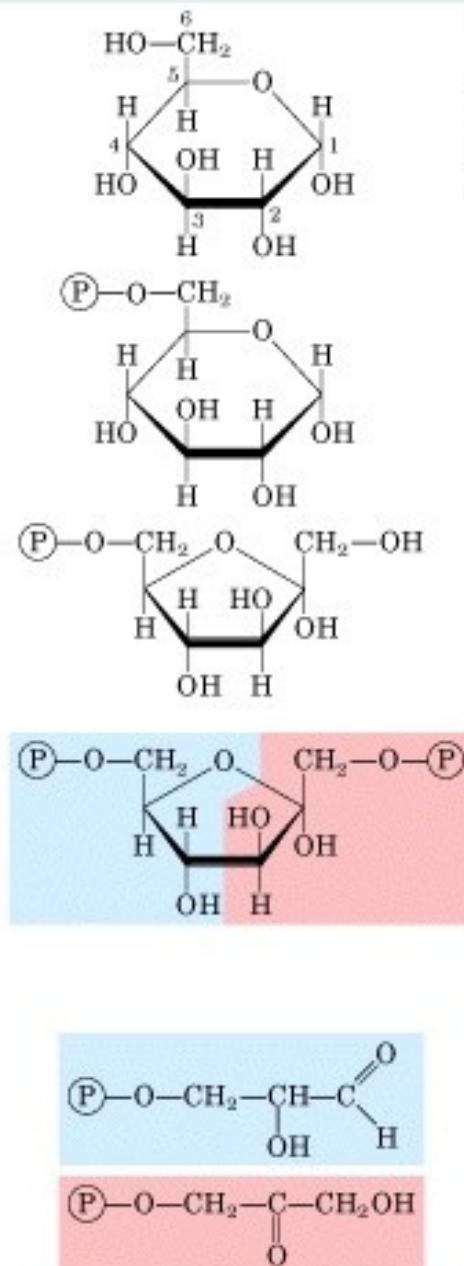
- 1. Possession of negative charge which inhibit their diffusion through membrane.**
- 2. Conservation of free energy in high energy phosphate bond.**
- 3. Facilitation of catalysis.**

(a)



Preparatory phase

Phosphorylation of glucose and its conversion to glycerinaldehyde 3-phosphate

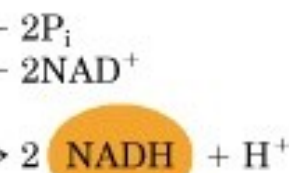


(b)

Glyceraldehyde 3-phosphate (2)

oxidation and phosphorylation

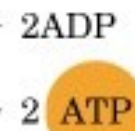
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1,3-Bisphosphoglycerate (2)

first ATP-forming reaction (substrate-level phosphorylation)

⑦



3-Phosphoglycerate (2)

⑧

2-Phosphoglycerate (2)

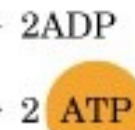
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Phosphoenolpyruvate (2)

second ATP-forming reaction (substrate-level phosphorylation)

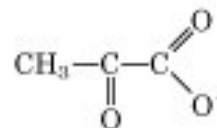
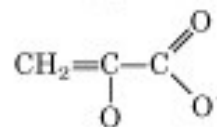
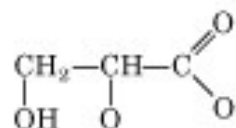
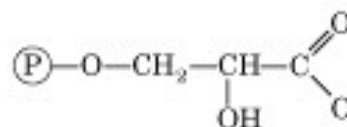
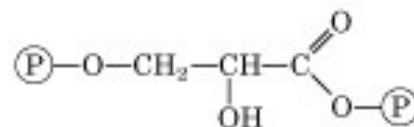
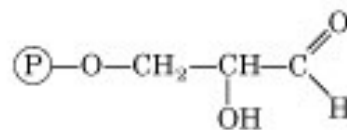
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Pyruvate (2)

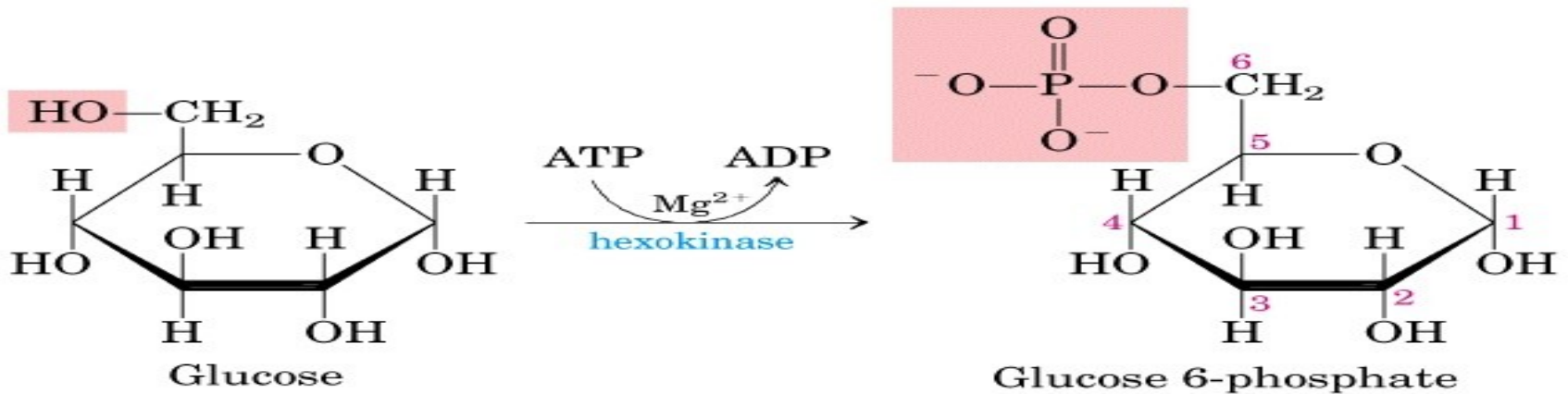
Payoff phase

Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH



1. Hexokinase reaction: Phosphorylation of hexoses (mainly glucose)

- I. This enzyme is present in most cells. In liver Glucokinase is the main hexokinase (both ISOENZYMES) which prefers glucose as substrate.
- II. It requires Mg-ATP complex as substrate. Un-complexed ATP is a potent competitive inhibitor of this enzyme.
- III. Enzyme catalyses the reaction by proximity effect; bringing the two substrate in close proximity.
- IV. This enzyme undergoes large conformational change upon binding with Glucose. It is inhibited allosterically by G6P.



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

Difference between Hexokinase & Glucokinase

Km low, high affinity for glu

Km high, low affinity for glu

Non-specific, can phosphorylate any of hexoses

specific, can phosphorylate only glucose

Present in tissues, supply glucose to tissues even in low blood glucose concentration

Present in liver only, it removes glucose from blood after meal.

Not effected by insulin

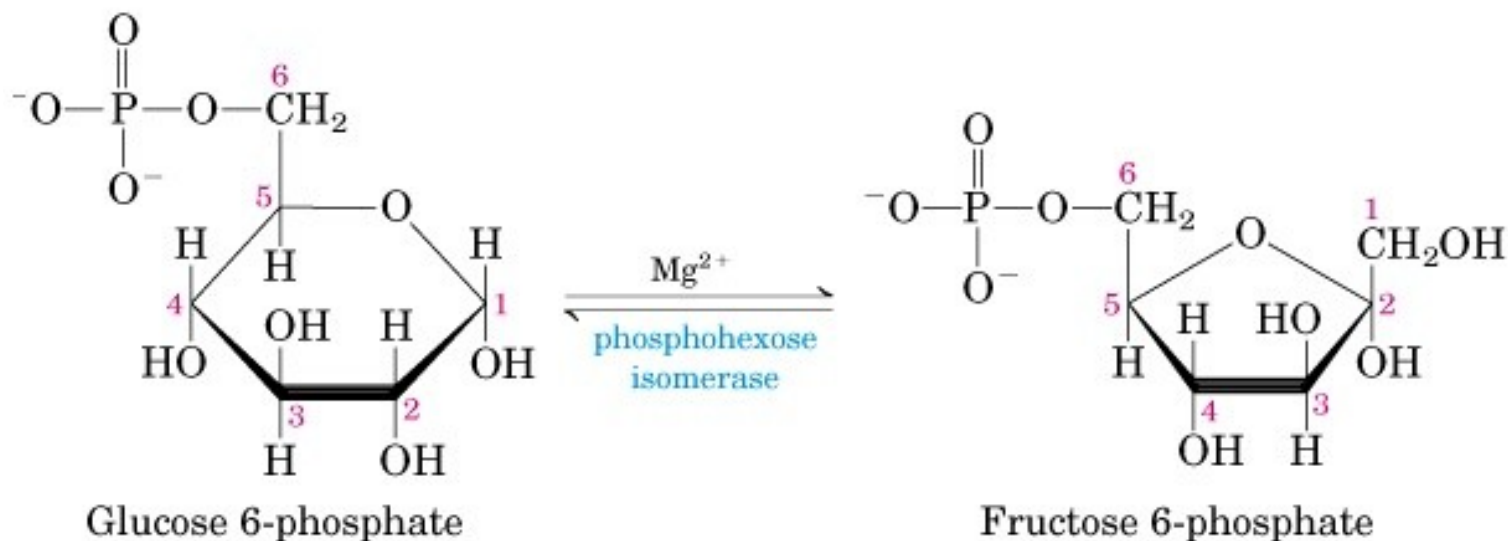
Stimulated by glucose and insulin

Allosterically inhibited by glucose

Not inhibited by glu-6-PO₄

2. Phosphoglucose Isomerase or Phosphohexose Isomerase: Isomerization of G6P to Fructose 6 phosphate.

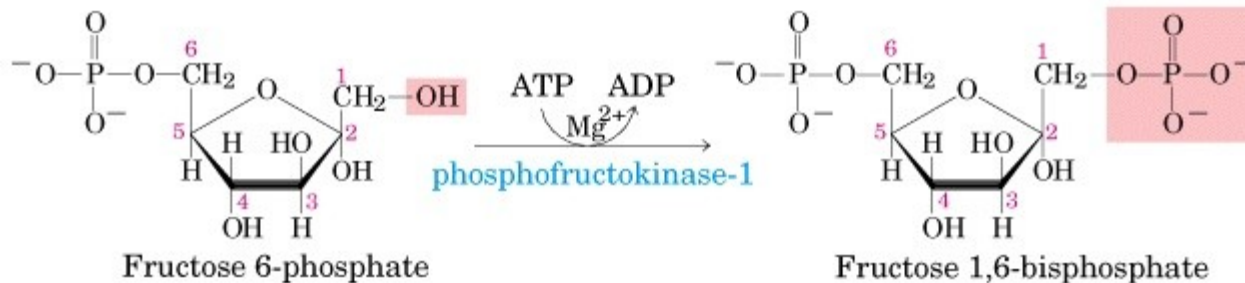
- I. This enzyme catalyzes the reversible isomerization of G6P (an aldohexose) to F6P (a ketohexose).
- II. This enzyme requires Mg^{++} for its activity.
- III. It is specific for G6P and F6P.



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

3. Phosphofructokinase-1 Reaction: Transfer of phosphoryl group from ATP to C-1 of F6P to produce Fructose 1,6 bisphosphate.

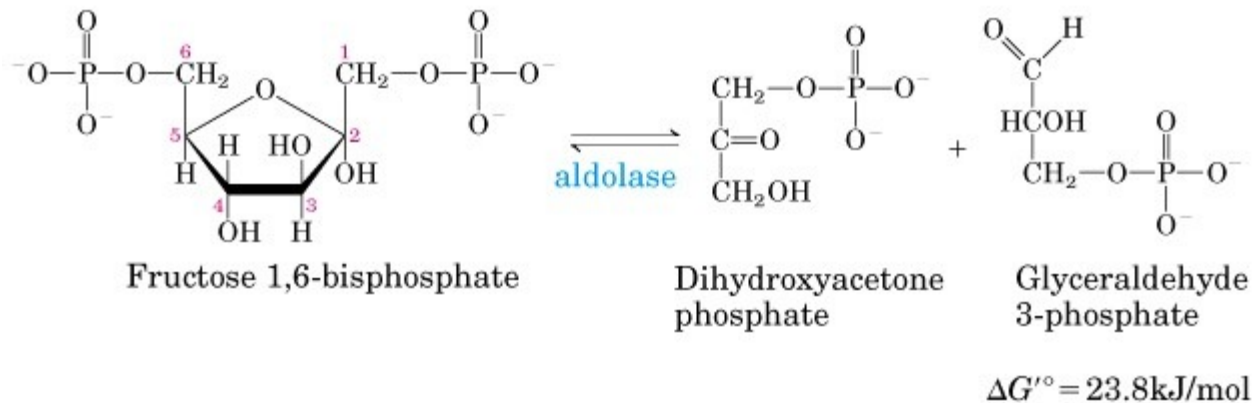
- I. This step is an important irreversible, regulatory step.
- II. The enzyme Phosphofructokinase-1 is one of the most complex regulatory enzymes, with various allosteric inhibitors and activators.
- III. ATP is an allosteric inhibitor, and Fructose 2,6 biphosphate is an activator of this enzyme.
- IV. ADP and AMP also activate PFK-1 whereas citrate is an inhibitor.



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

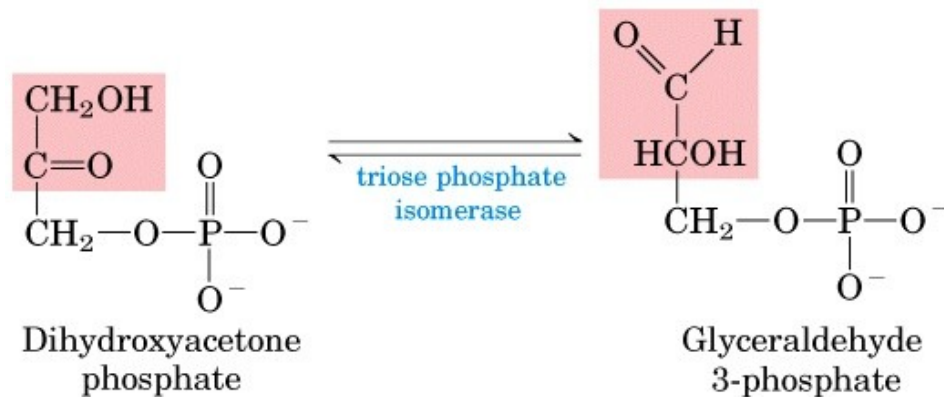
4. Aldolase Reaction: Cleavage of Fructose 1,6 bisphosphate into glyceraldehyde 3 phosphate (an aldose) and dihydroxy acetone phosphate (a ketose).

- I. This enzyme catalyses the cleavage of F1,6 biphosphate by aldol condensation mechanism.
- II. As shown below, 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.



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

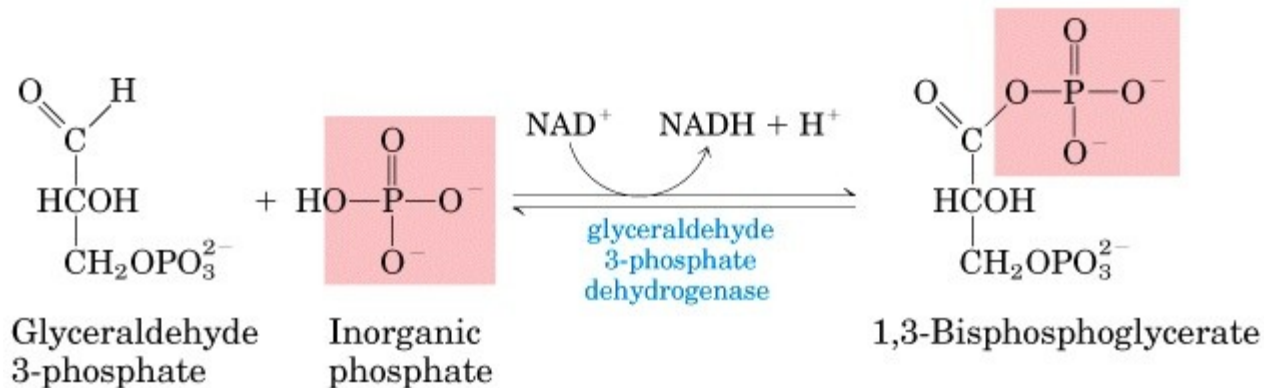
I. This a reversible reaction catalysed by acid-base catalysis in which Histidine-95 and Glutamate -165 of the enzyme are involved.



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

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

- I. This is the first reaction of energy yielding step. Oxidation of aldehyde derives the formation of a high energy acyl phosphate derivative.
- 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. The free energy released in the oxidation reaction is used in the formation of acylphosphate.



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

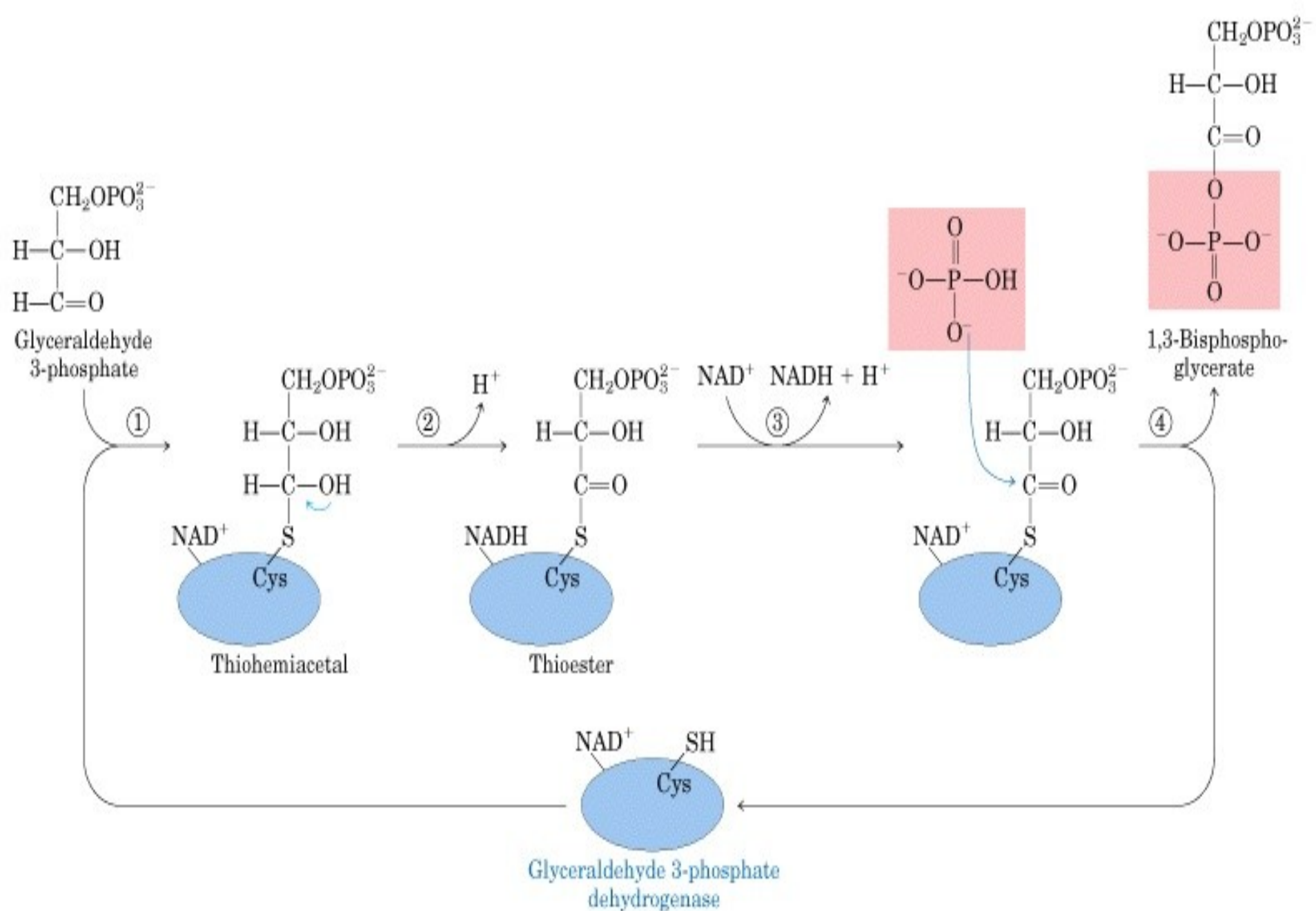
The mechanism of GAPDH reaction:

Evidence for the mechanism;

- I. Iodoacetate inhibits this reaction, indicating the involvement of Cysine residue of enzyme.
- II. Tritium from GAP is transferred to NAD, indicating transfer of hydride ion in oxidation reaction.
- III. ^{32}P exchanges with PO_4^{--} indicating acyl enzyme intermediate.

Steps in reaction mechanism:

1. Glycerinaldehyde- 3- phosphate (**GAP**) binding to the enzyme.
2. Nucleophilic attack by SH group (sulfhydryl group) on CHO group forming a thiohemiacetal.
3. Direct transfer of hydride to NAD^+ leading to the formation of thioester. Energy of this oxidation is conserved in synthesis of thioester and NADH.
4. Another molecule of NAD^+ replaces NADH from enzyme site.
5. Nucleophilic attack on thioester by PO_4^{--} to form 1,3 bisphosphoglycerate



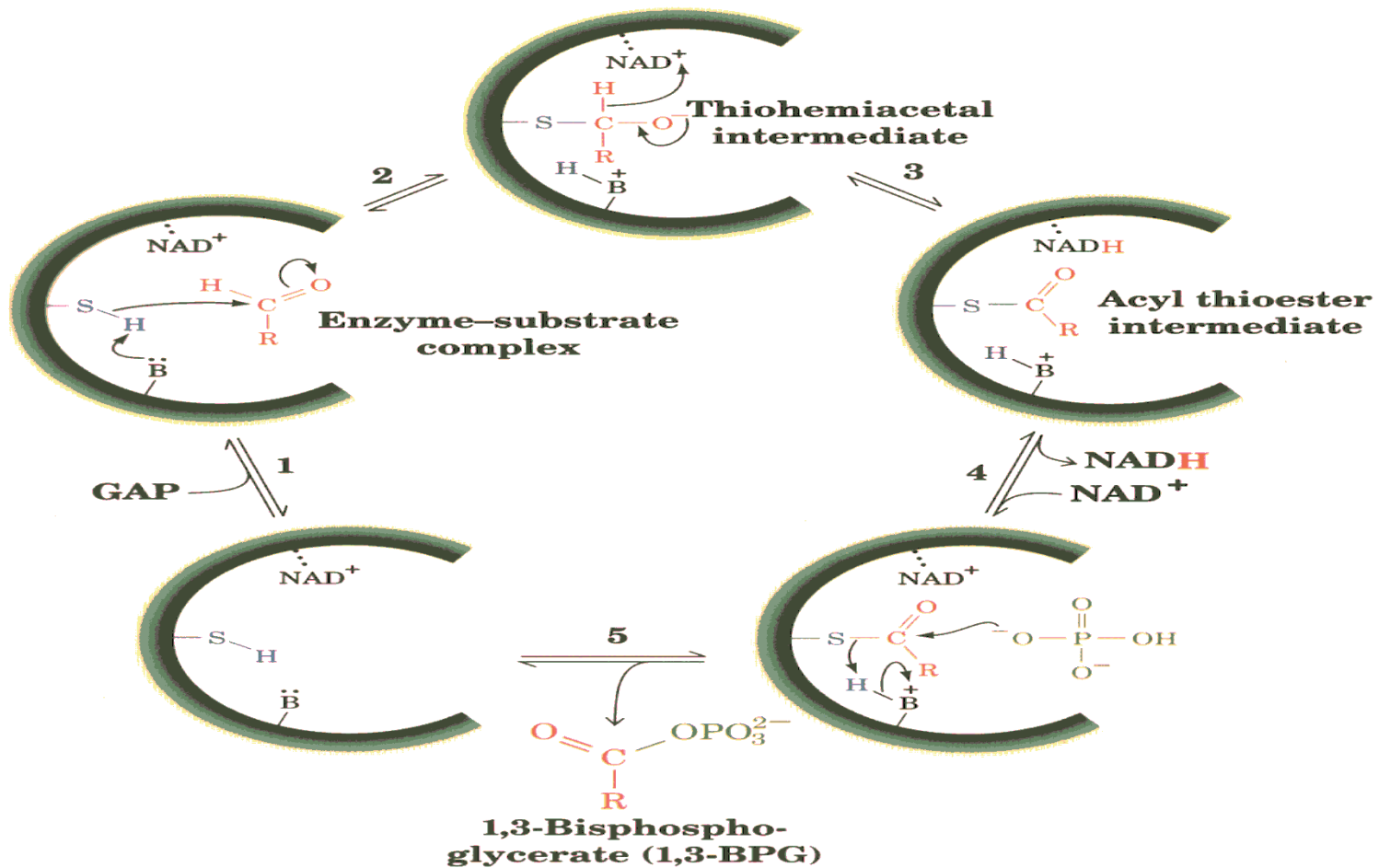
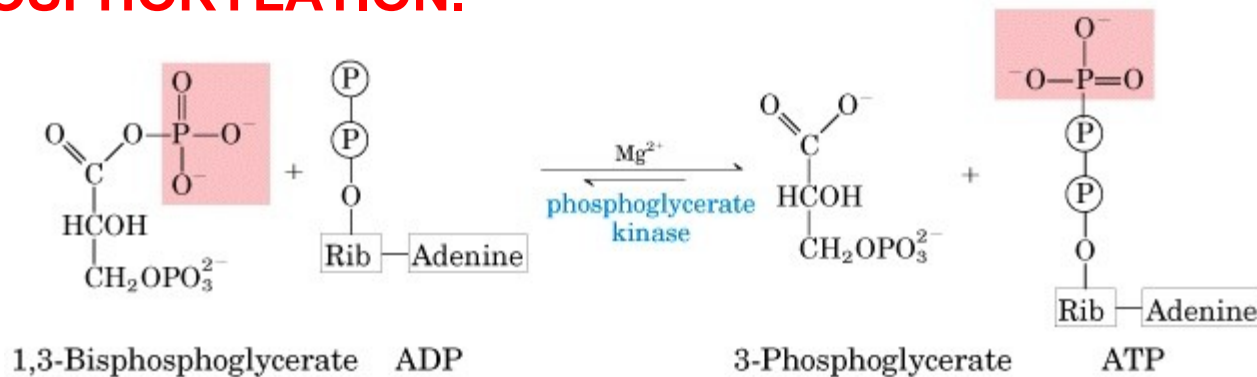


Figure 16-11. Mechanism of glyceraldehyde-3-phosphate dehydrogenase.

7. Phosphoglycerate kinase Reaction: Transfer of phosphoryl group from 1,3 bisphosphoglycerate to ADP generating ATP.

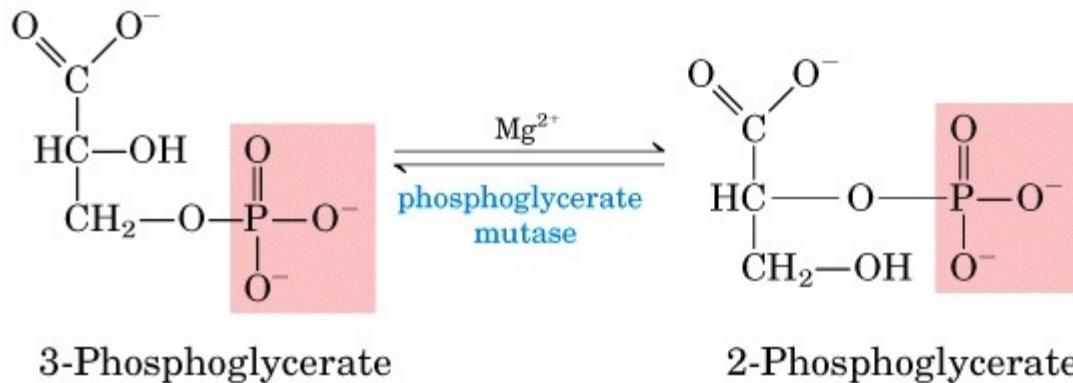
- I. The name of this enzyme indicates its function for reverse reaction.
- II. It catalyses the formation by proximity effect. ADP-Mg bind on one domain and 1,3BPG binds on the other and a conformational change brings them together similar to hexokinase.
- III. This reaction and the 6th step are coupled reaction generating ATP from the energy released by oxidation of 3-phosphoglycerate.
- IV. This step generates ATP by SUBSTRATE-LEVEL PHOSPHORYLATION.



$$\Delta G'^{\circ} = -18.5 \text{ kJ/mol}$$

8. Phosphoglycerate Mutase Reaction: Conversion of 3-phosphoglycerate to 2-phosphoglycerate (2-PG).

- I. In active form, the phosphoglycerate mutase is phosphorylated at His-179.
- II. There is transfer of the phosphoryl group from enzyme to 3-PG, generating enzyme bound 2,3-bisphosphoglycerate intermediate. This compound has been observed occasionally in reaction mixture.
- III. In the last step of reaction the phosphoryl group from the C-3 of the intermediate is transferred to the enzyme and 2-PG is released.
- IV. In most cells 2,3BPG is present in trace amount, but in erythrocytes it is present in significant amount. There it regulates oxygen affinity to hemoglobin.



$$\Delta G'^{\circ} = 4.4 \text{ kJ/mol}$$

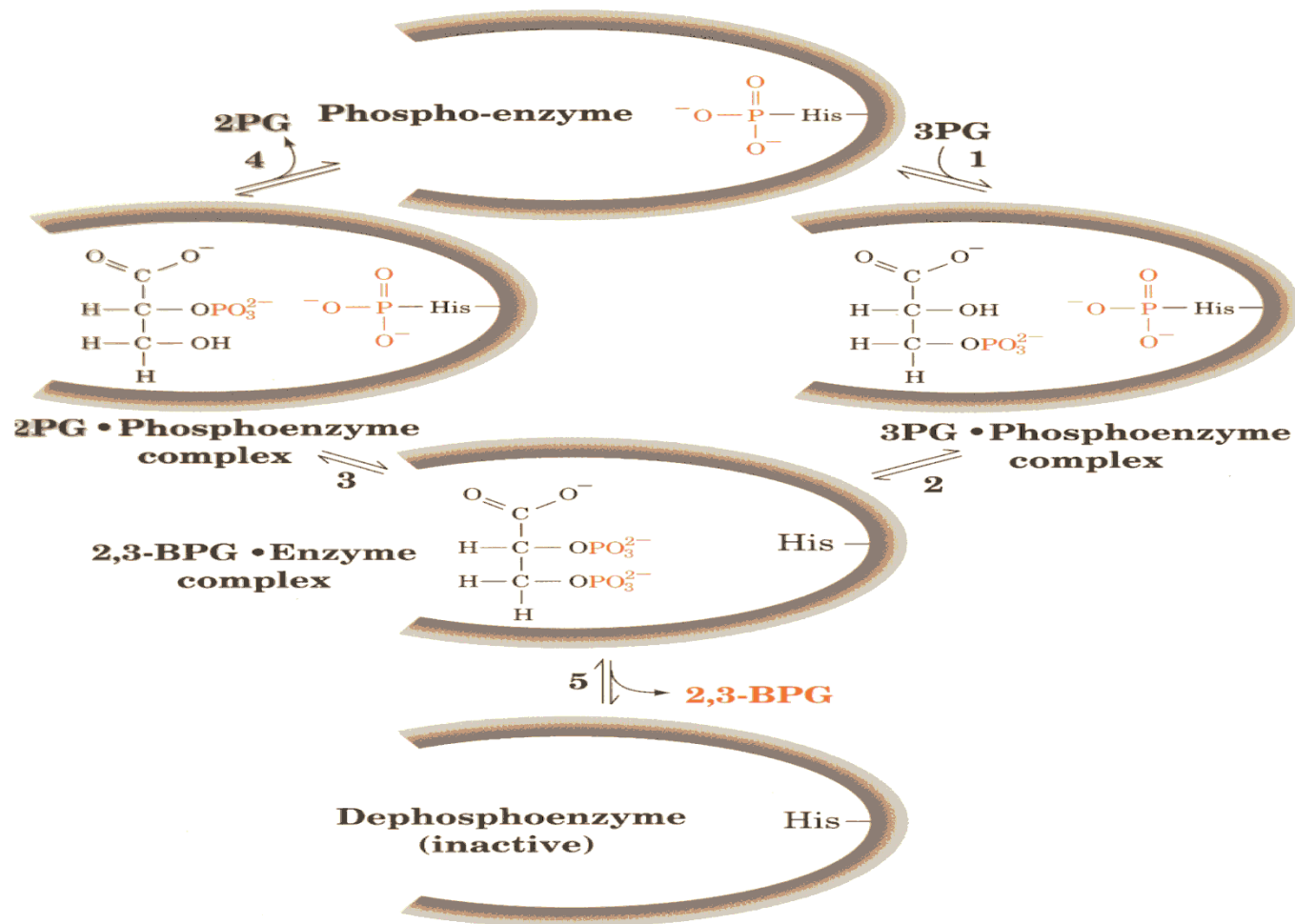
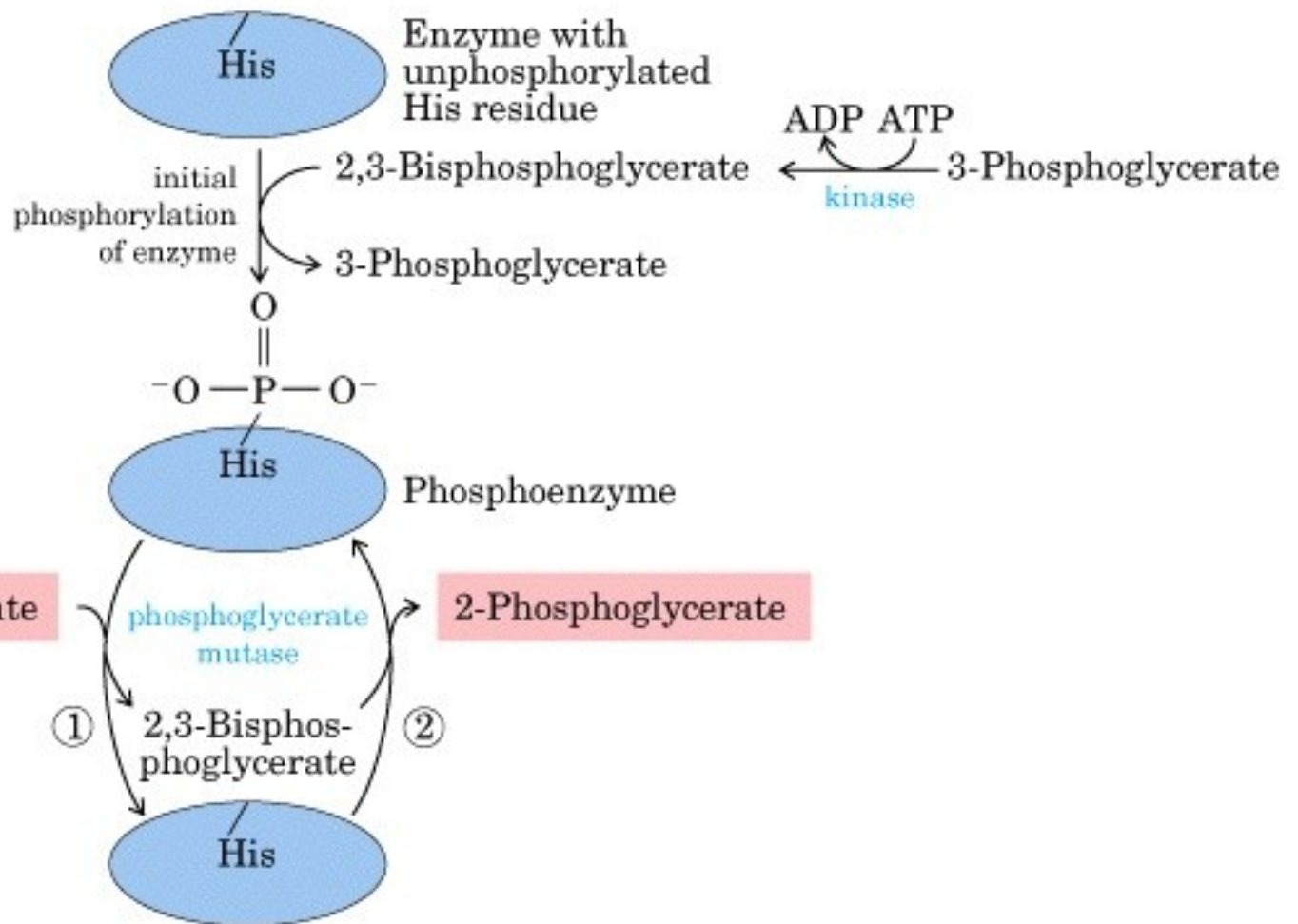
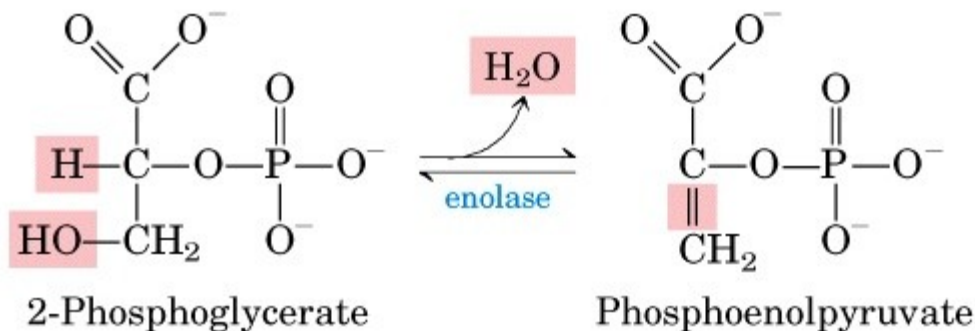


Figure 16-15. Mechanism of phosphoglycerate mutase.



9. Enolase Reaction: Dehydration of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP).

- I. Dehydration of 2-PG by this reaction increases the standard free energy change of hydrolysis of phosphoanhydride bond.
- II. Mechanism: Rapid extraction of proton from C-2 position by a general base on enzyme, generating a carbanion. The abstracted proton is readily exchanged with solvent.
- III. The second rate limiting step involves elimination of OH group generating PEP



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

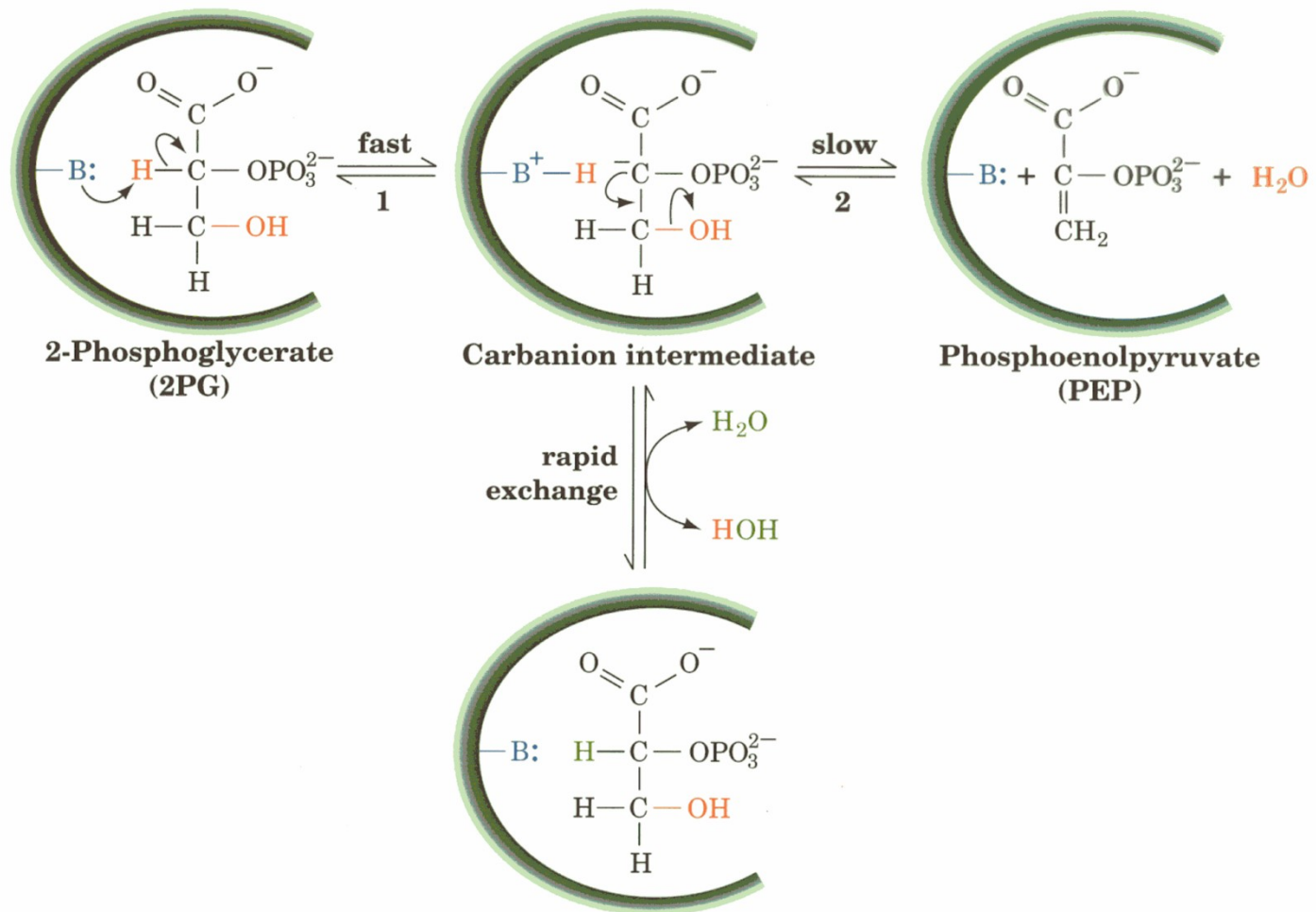
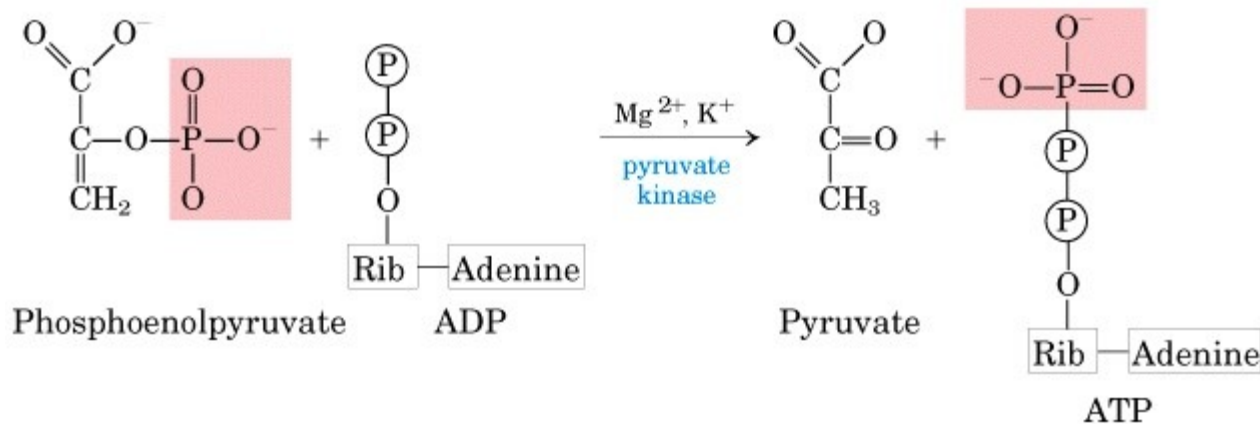


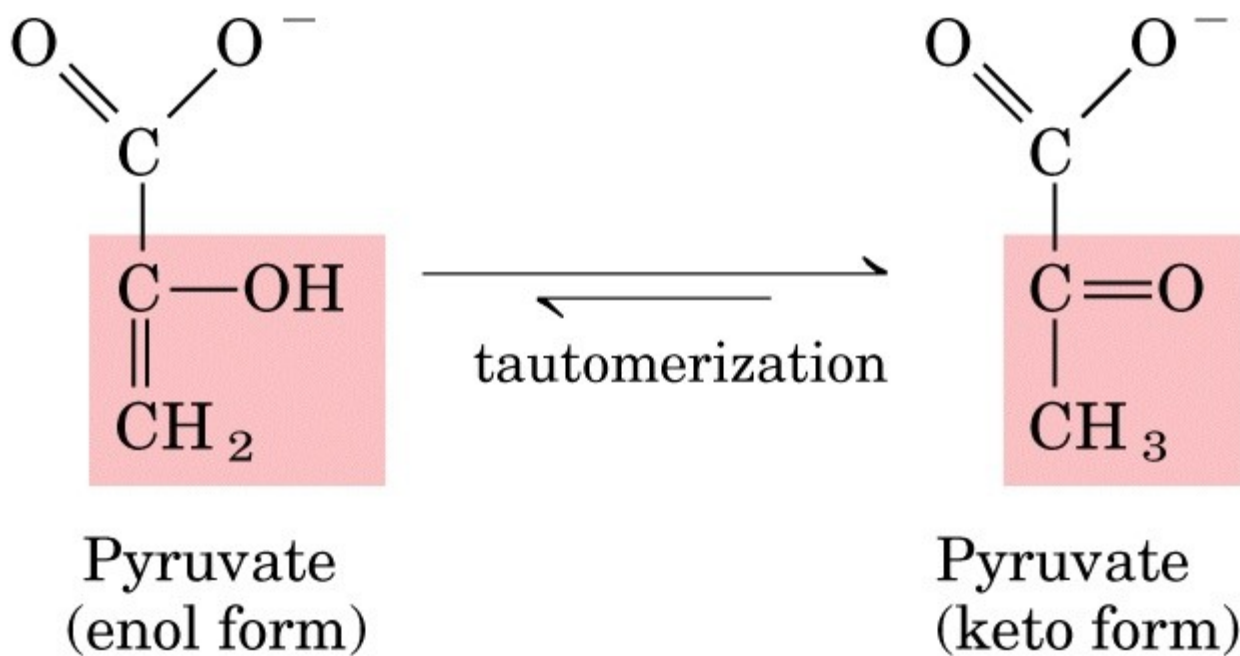
Figure 16-18. Mechanism of enolase.

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

- I. This is the second substrate level phosphorylation reaction of glycolysis.
- II. This enzyme couple the free energy of PEP hydrolysis to the synthesis of ATP
- III. This enzyme requires Mg^{++} and K^+



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



Pyruvate
(enol form)

Pyruvate
(keto form)

Energetics and products of Glycolysis:

From one molecule of Glucose:

$1\text{Gl} + 2\text{ATP} + 2\text{NAD}^+ + 4\text{ADP} + 4\text{P}_i = 2\text{pyruvate} + 2\text{NADH} + 4\text{ATP} + 2\text{ADP} + 2\text{P}_i$

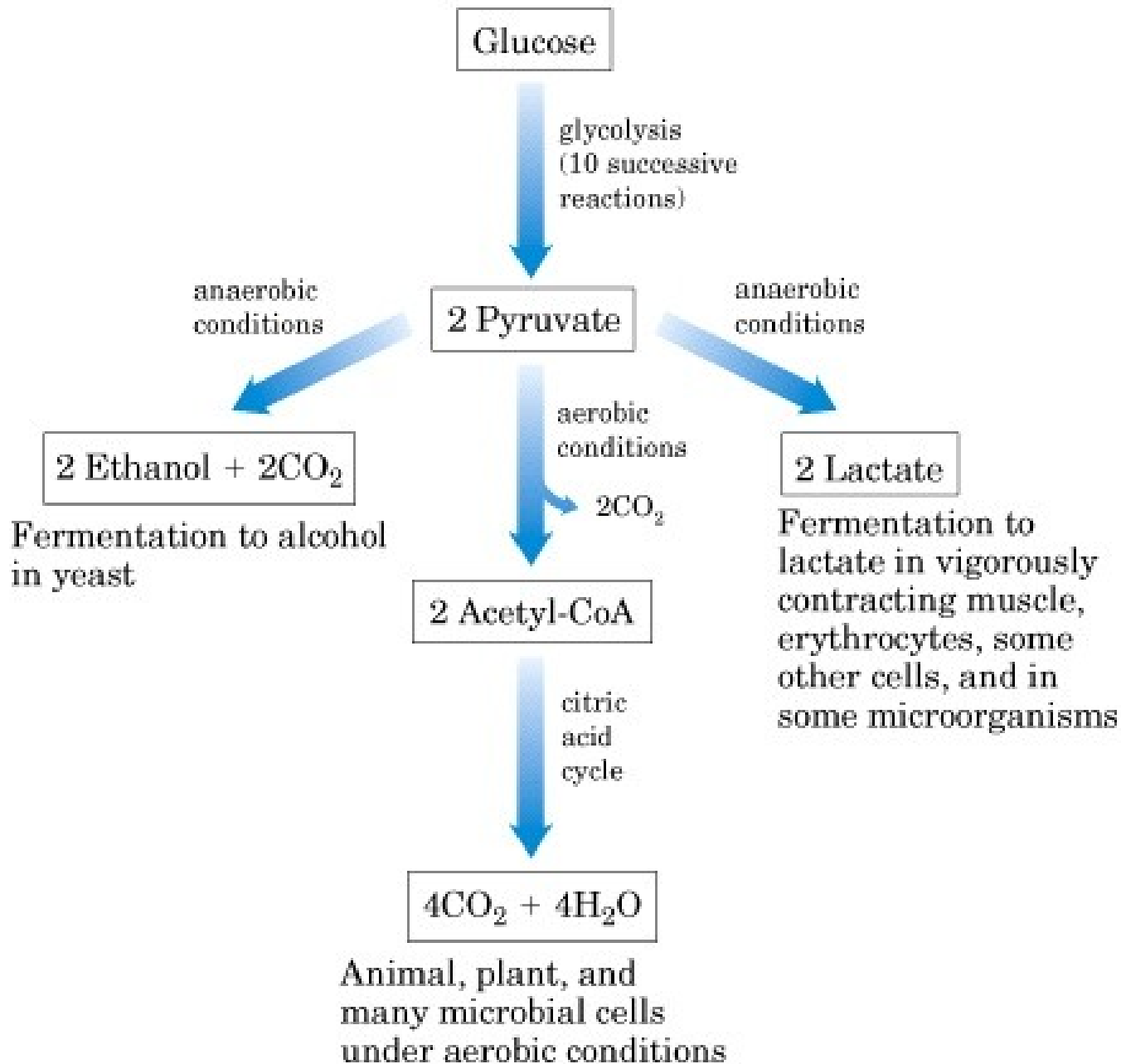
After balancing: $1\text{Gl} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_i = 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH}$

2 molecules of ATP generated can directly be used for doing work or synthesis.

The 2 NADH molecules are oxidized in mitochondria under aerobic condition and the free energy released is enough to synthesize 6 molecules of ATP by oxidative phosphorylation.

Under the aerobic condition, pyruvate is catabolized further in mitochondria through pyruvate dehydrogenase and cytric acid cycle where all the carbon atoms are oxidized to CO₂. The free energy released is used in the synthesis of ATP, NADH and FADH₂.

Under anaerobic condition: Pyruvate is converted to Lactate in homolactic fermentation or in ethanol in alcoholic fermentation.



Energetics of Glycolysis Pathway

ATP FORMED:

1. Gly-3-PO₄ → 1,3 Bisphosphoglycerate = 6 ATP
2. 1,3 Bisphosphoglycerate → 3-Phosphoglycerate = 2 ATP
3. Phosphoenolpyruvate → Enol pyruvate = 2 ATP

ATP CONSUMED:

4. Glucose → Glucose-6-PO₄ = 1 ATP
5. Fru-6-PO₄ → Fru-1,6 bisphosphate = 1ATP

Net ATP synthesized

10 – 2 = 8 ATP

Effect of hormones in glycolysis

- 1. Insulin stimulate Hexokinase & Glucokinase by converting glucose to glu-6-PO₄**
- 2. Insulin stimulate Phosphofructokinase converting fru-6-PO₄ to Fru-1,6 bisphosphate**
- 3. Glucagon stimulate liver glu-6-PO₄ by converting glu-6-PO₄ to glucose & fru-1,6- bisphosphate.**
- 4. Fru-1,6- bisphosphate is converted to fru-6-PO₄**

INHIBITORS

1. **Iodoacetate** inhibit **Gly-3-PO₄** dehydrogenase involved in gly-3-PO₄ to 1,3-bisphosphoglycerate
2. **Arsenate** inhibit synthesis of ATP in the conversion of 1,3 bisphosphoglycerate to 3-phosphoglycerate.
3. **Fluoride** inhibit enolase in conversion of 2-Phosphoglycerate to phosphoglycerate

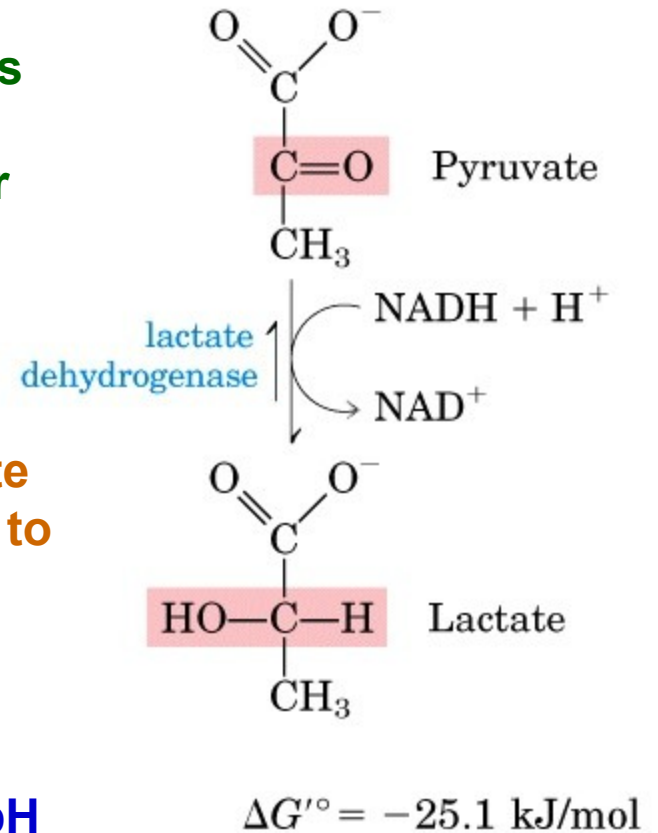
Homolactic Fermentation:

In an anaerobic condition or in the need of sudden need of high amount of ATP, glycolysis is the main source for generation of ATP.

NAD^+ is one of the crucial cofactor required for GAPDH reaction. In order to regenerate NAD^+ from the reduced form (NADH), this reaction takes place in muscle cells.

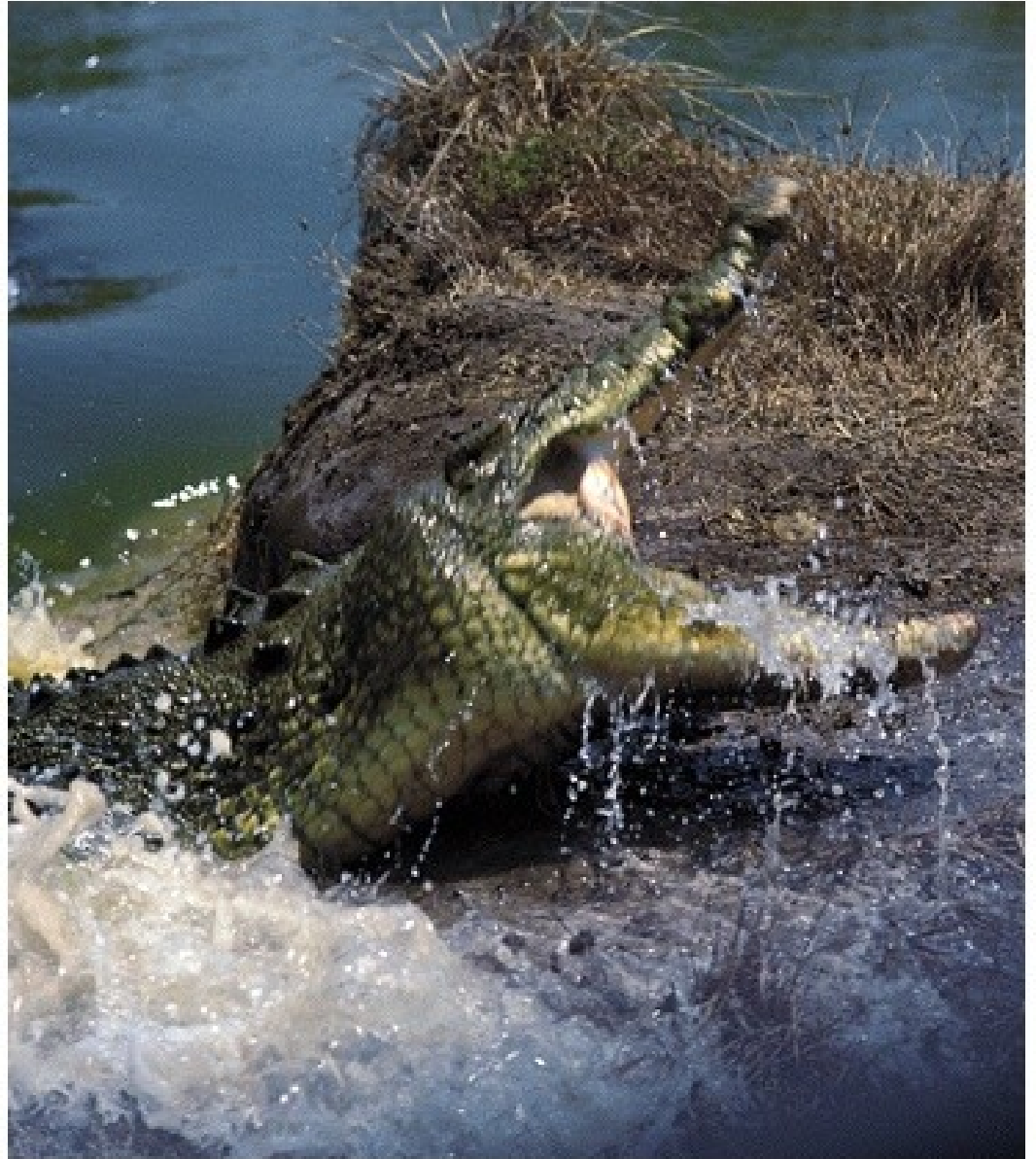
Lactate dehydrogenase (LDH) reduces pyruvate to lactate using NADH and thereby oxidizing it to NAD^+ .

Other than regenerating NAD^+ for running GAPDH reaction, LDH reaction is a waste of energy, and its product lactic acid brings the pH lower and causes fatigue.



Glycolysis can generate sudden burst of ATP without oxygen, using glucose and glycogen storage of muscle and liver.

NAD⁺ is regenerated by lactic fermentation to carry out GAPDH reaction of glycolysis.



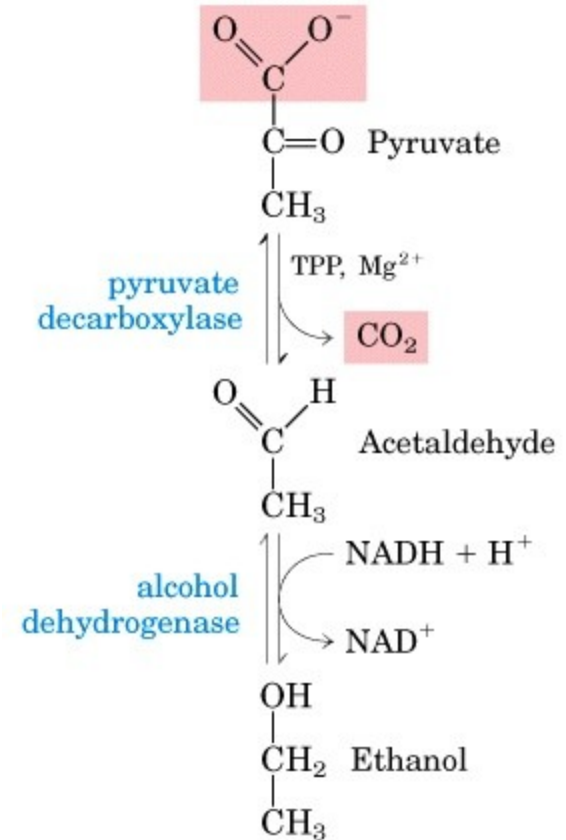
Alcoholic fermentation:

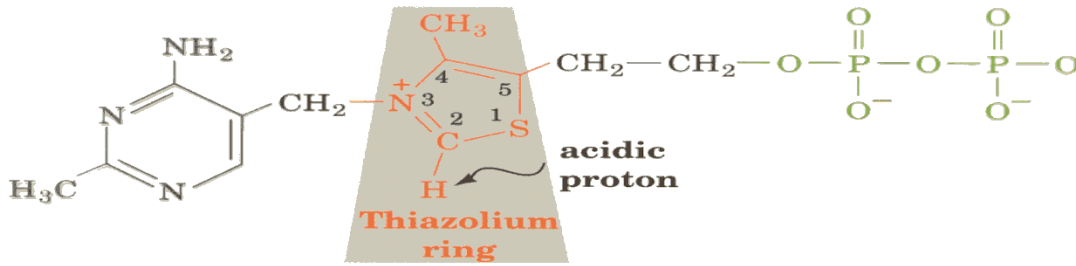
Microorganisms and yeast convert pyruvate to alcohol and carbon dioxide to regenerate NAD^+ for glycolysis (step 6, GAPDH).

It is a two step process:

1. **Pyruvate decarboxylase (PDC) reaction:** This enzyme is Mg^{++} -dependent and requires an enzyme-bound cofactor, thiamine pyrophosphate (TPP). In this reaction a molecule of CO_2 is released producing acetaldehyde.

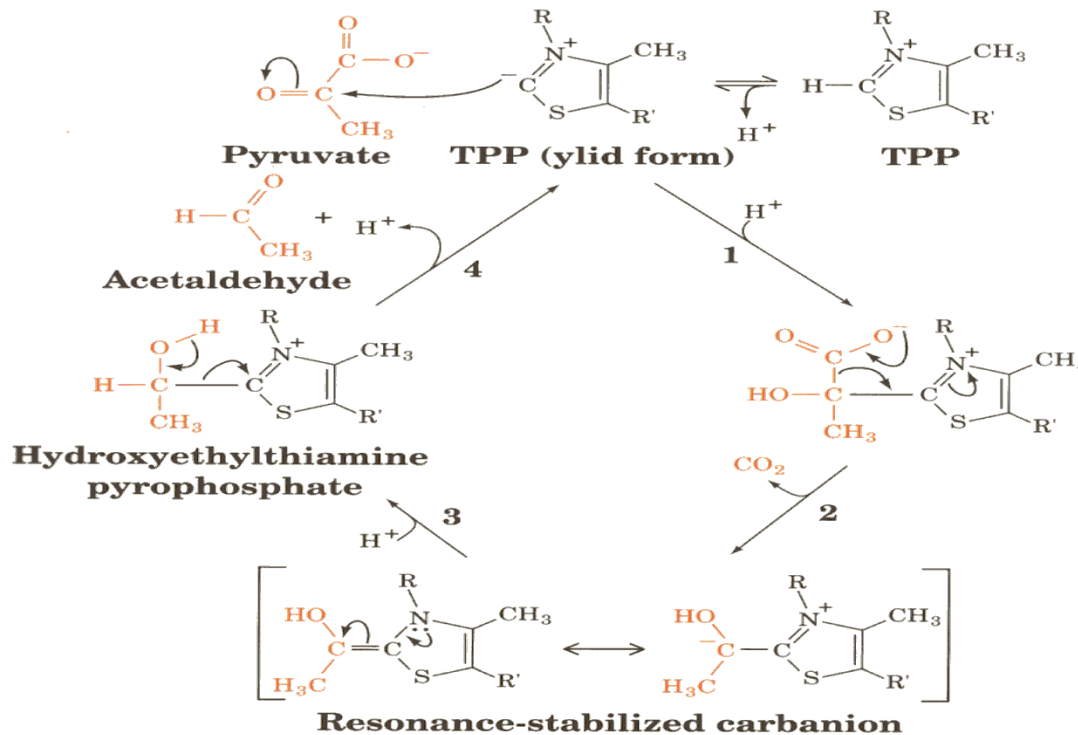
2. **Alcohol dehydrogenase reaction:** Acetaldehyde is reduced to ethanol using NADH as reducing power, thus regenerating NAD^+ .





Reaction mechanism of PDC

Figure 16-24. Thiamine pyrophosphate (TPP).



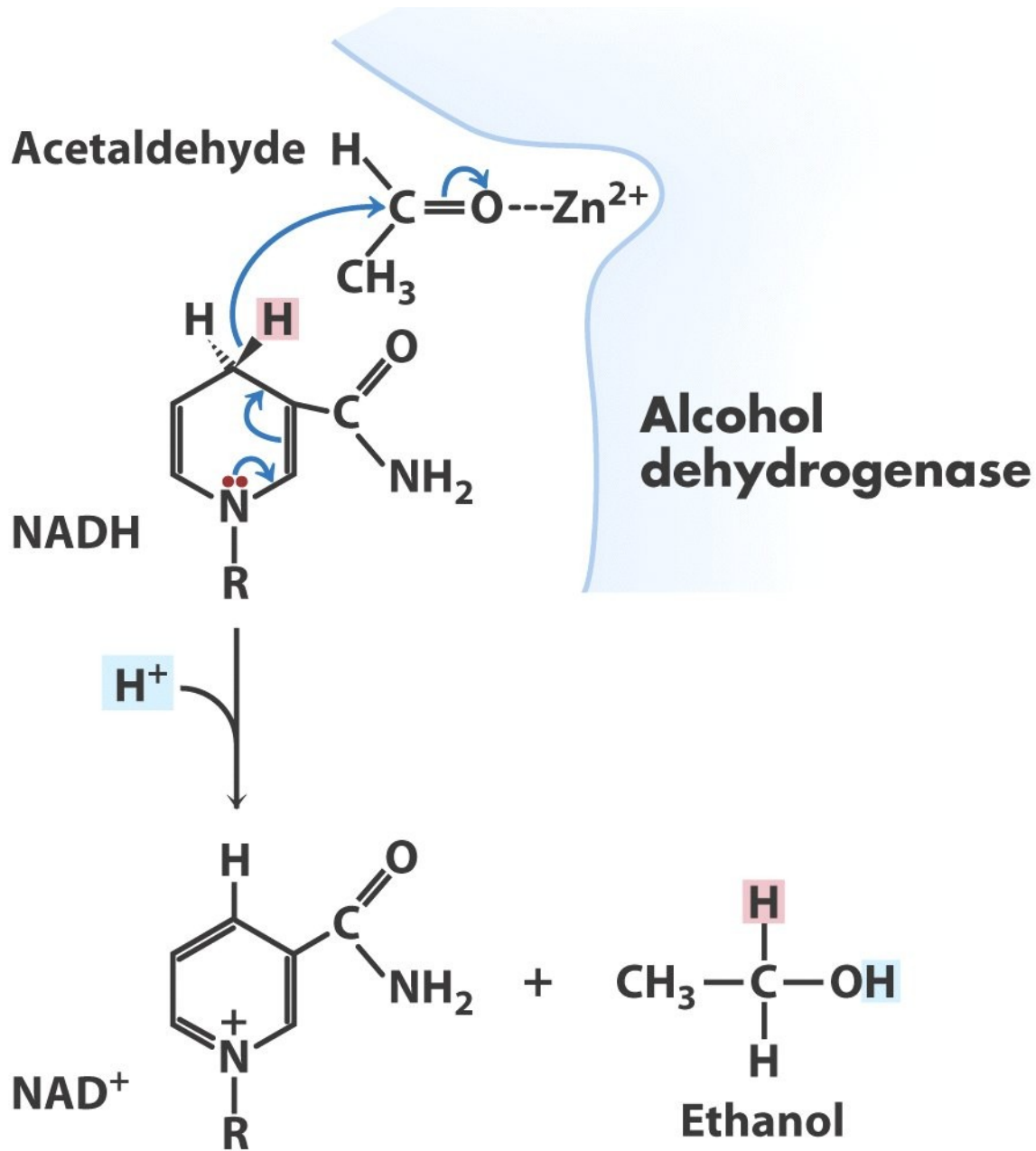
I. Nucleophilic attack on carbonyl carbon of pyruvate by TPP anion.

II. Departure of CO₂ leaving the carbanion-TPP adduct.

III. Protonation of carbanion

IV. Release of acetaldehyde following regeneration of TPP

Figure 16-25. Mechanism of pyruvate decarboxylase.



Regulation of Glycolysis:

Two types controls for metabolic reactions:

- a) **Substrate limited** : When concentrations of reactant and products in the cell are near equilibrium, then it is the availability of substrate which decides the rate of reaction.

- b) **Enzyme-limited**: When concentration of substrate and products are far away from the equilibrium, then it is activity of enzyme that decides the rate of reaction. These reactions are the one which control the flux of the overall pathway.

There are three steps in glycolysis that have enzymes which regulate the flux of glycolysis.

- I. **The hexokinase (HK)**
- II. **The phosphofruktokinase (PFK)**
- III. **The pyruvate kinase**

Phosphofructokinase-1 (PFK-1):

It's activity is controlled by a complex allosteric regulation.
This reaction commits the cells to channel glucose to glycolysis.

ATP is the end product of glycolysis as well as it is substrate for PFK-1.
In presence of high concentration of ATP, ATP binds to inhibition site of PFK, and thereby decreases the activity of enzyme.

AMP, ADP and Fructose 2, 6 biphosphate act as allosteric activators of this enzyme.

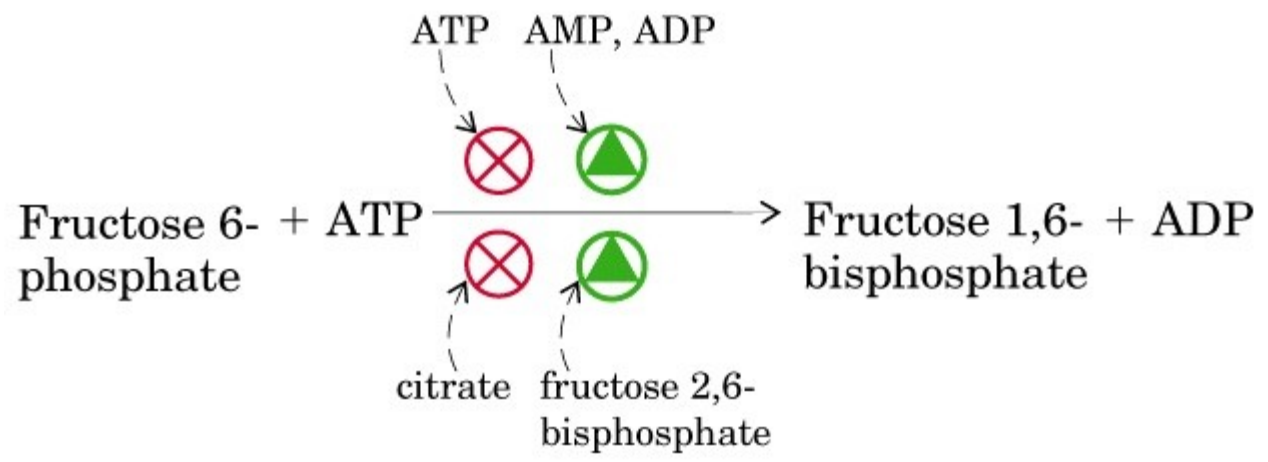
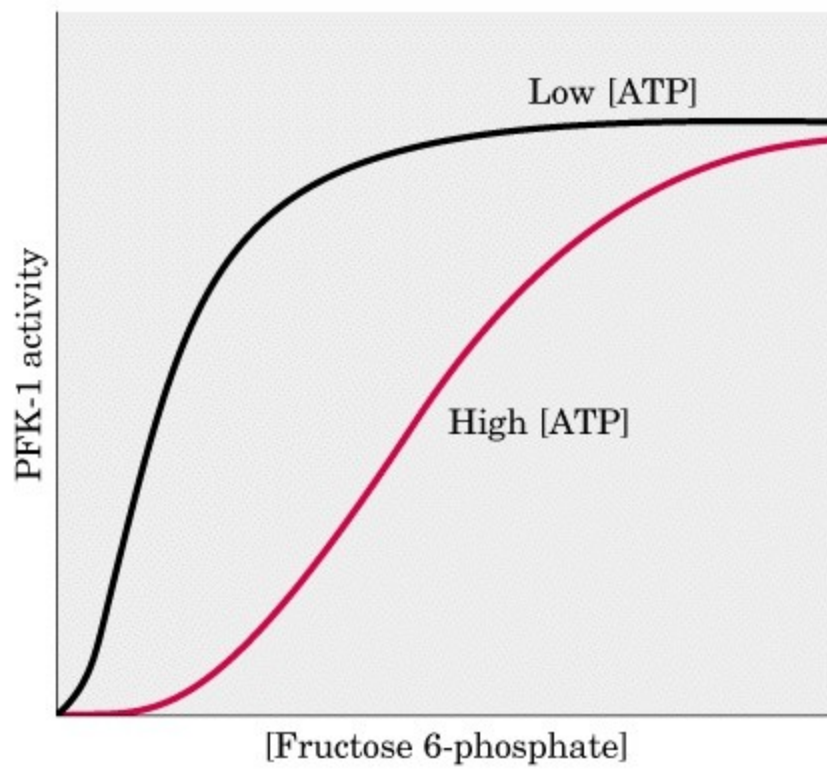
Activation of enzyme by AMP overcomes the inhibitory effect of ATP.

Two other enzymatic activities are involved in the regulation of PFK.

a) **Adnylate kinase:** It readily equilibrates 2 ADP molecules to one ATP and 1 AMP: $2\text{ADP} = \text{ATP} + \text{AMP}$, $K = \frac{[\text{ATP}][\text{AMP}]}{[\text{ADP}]^2} = 0.44$

Any decrease in ATP and increase in ADP results in an increase in AMP concentration, which activates PFK.

b) **Fructose 1,6-bisphosphatase (FBPase):** It catalyzes conversion of FBP to Fructose 6-phosphate, thus reverting back the PFK reaction.



(c)

Substrate cycle or futile cycle: In order to control the flux of glycolysis and to have better regulation, cells have FBPase which keeps degrading the product of PFK reaction (FBP) to its substrate (F-6-P). This is called substrate cycle.

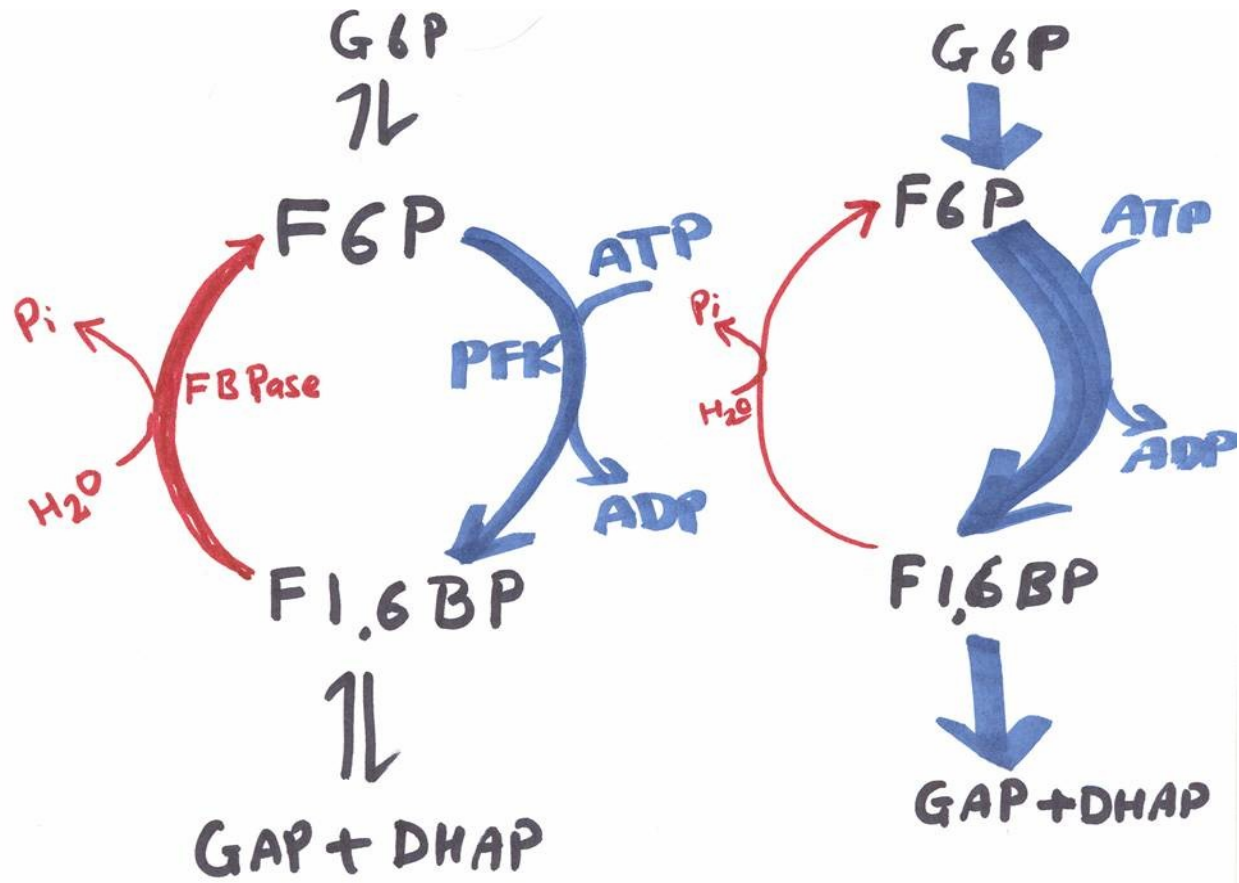
This is a futile exercise where, cells invest an ATP to produce FBP which is hydrolysed back to F6-P by FBPase. This is a price cells pay to keep glycolysis in check.

AMP acts as a potent inhibitor of FBPase. Thus the rate of glycolysis can be increased many fold by AMP as it activates PFK and at the same time it inhibits FBPase activity.

Hexokinase: It is allosterically inhibited by its product Glucose 6 phosphate. In liver Glucokinase is inhibited by Fructose 6 Phosphate. Uncomplexed ATP acts as a competitive inhibitor of this enzyme.

Pyruvate Kinase: It is allosterically inhibited by ATP. ATP binding to the inhibitor site of pyruvate kinase decreases its ability to bind to phosphoenol pyruvate (PEP) the substrate.

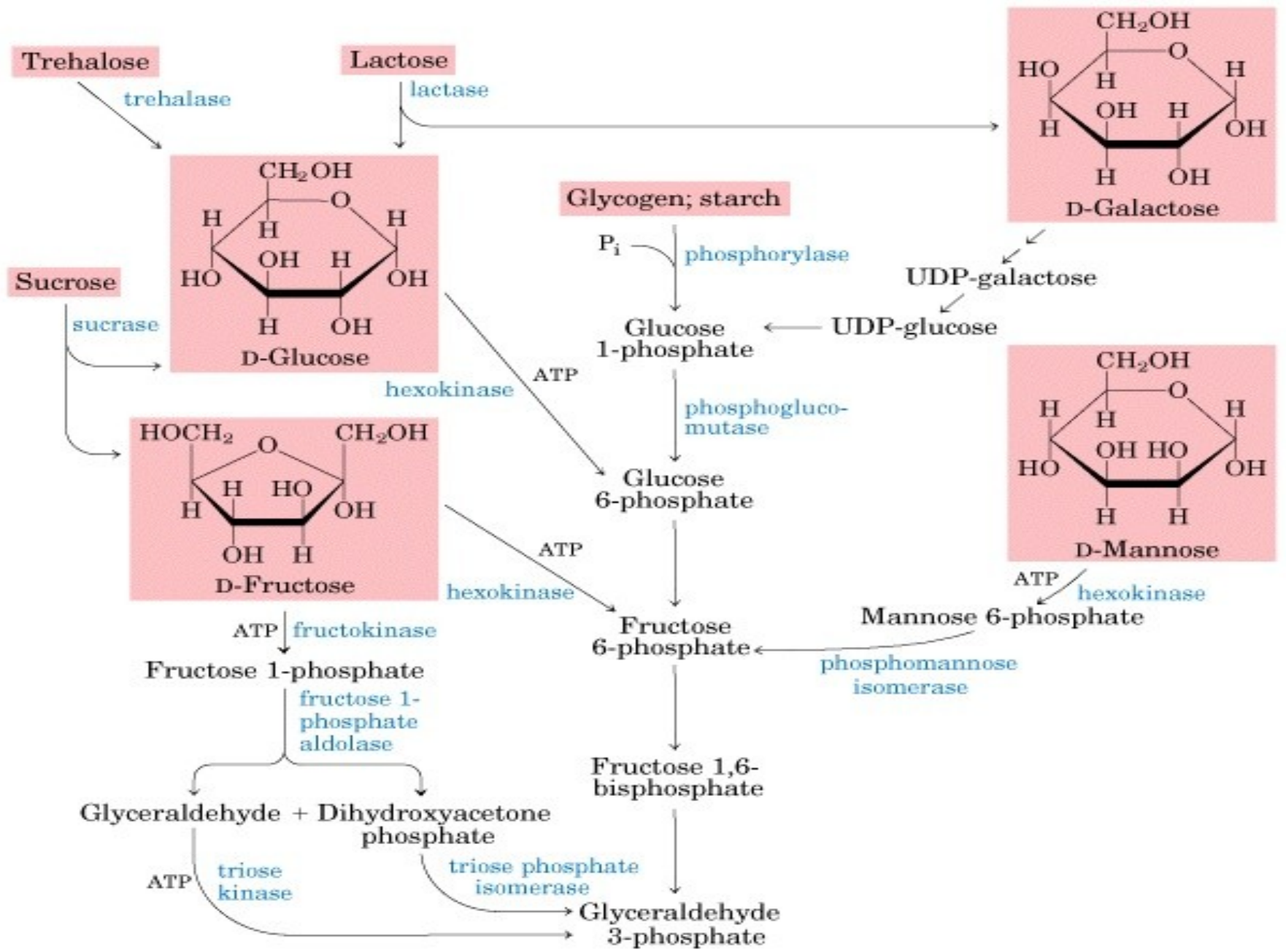
It is also inhibited by Acetyl coenzyme A and long chain fatty acid.

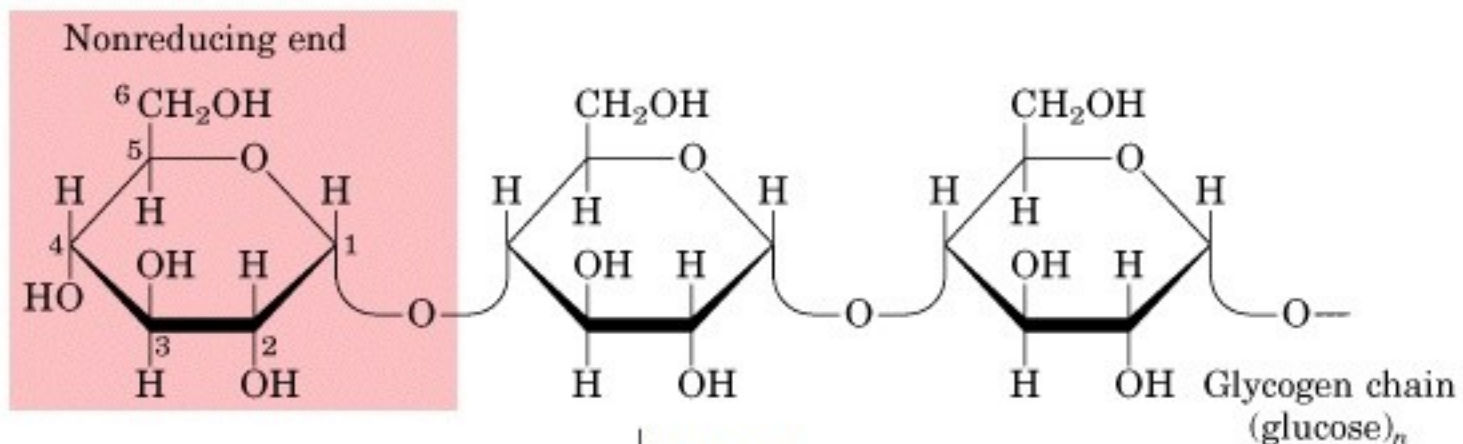


LOW AMP
HIGH ATP

High AMP
LOW ATP

SUBSTRATE CYCLE





P_i ↓ glycogen phosphorylase

