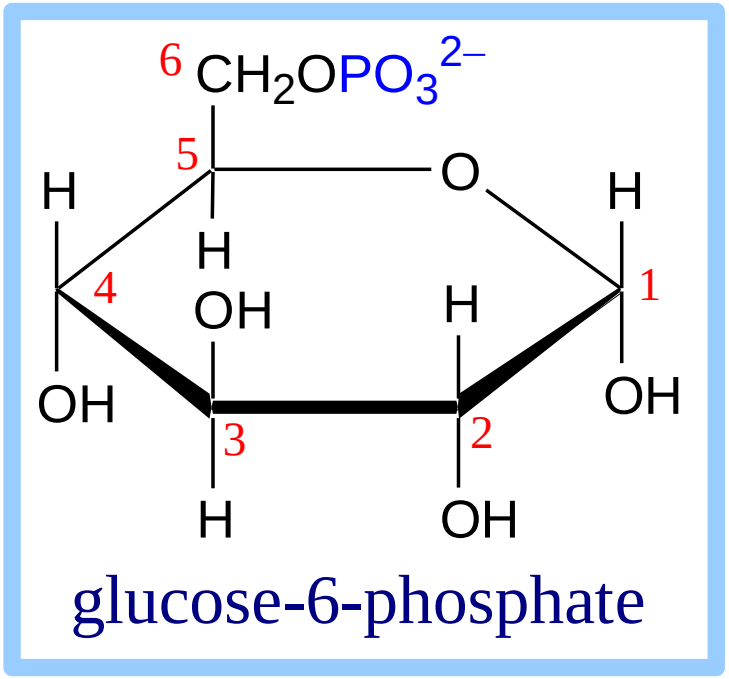
Glycolysis

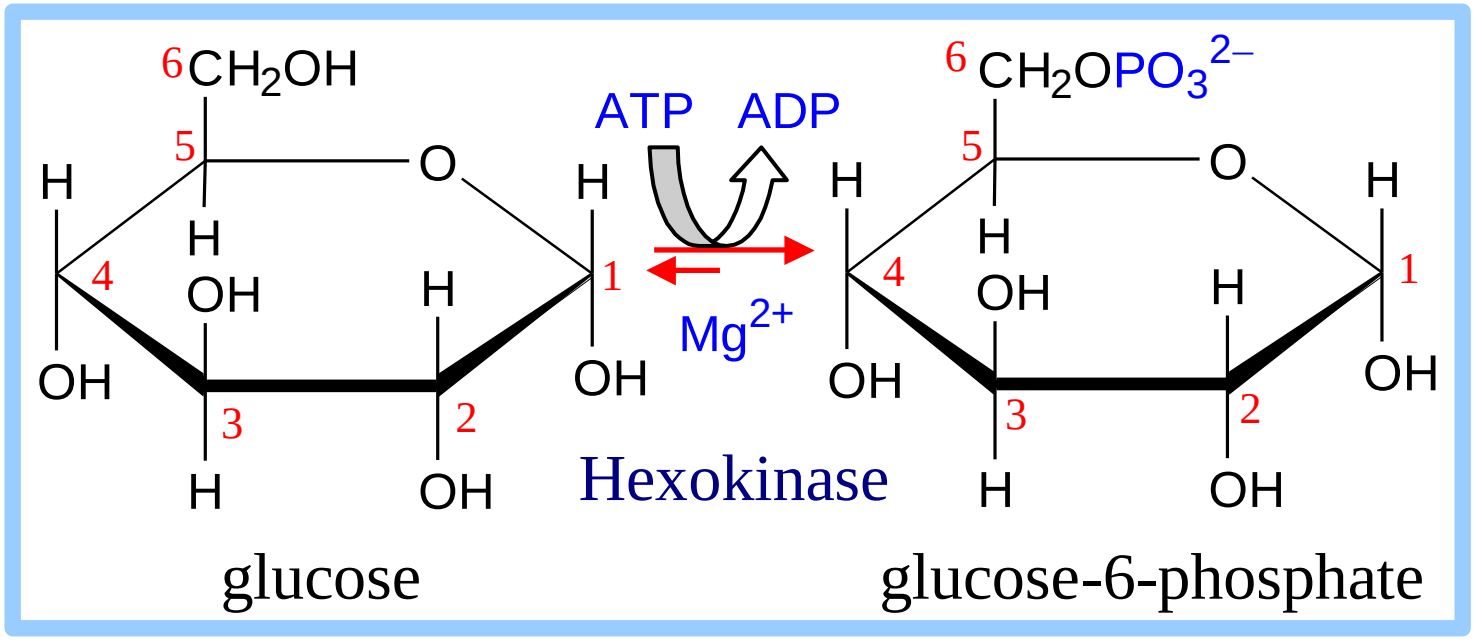


glucose-6-phosphate

Glycolysis takes place in the **cytosol** of cells.

Glucose enters the Glycolysis pathway by conversion to **glucose-6-phosphate**.

Initially there is energy input corresponding to cleavage of two ~P bonds of ATP.

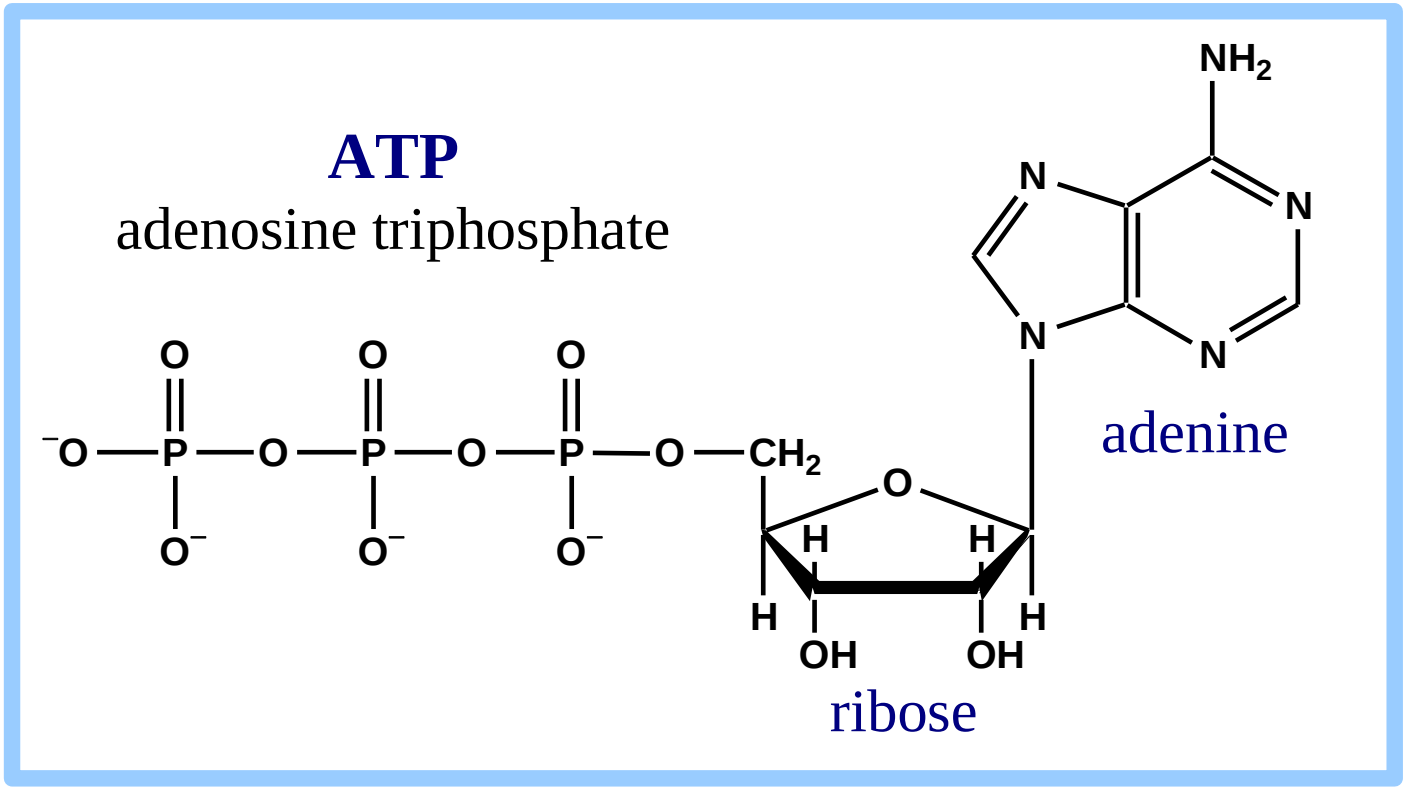


1. Hexokinase catalyzes:

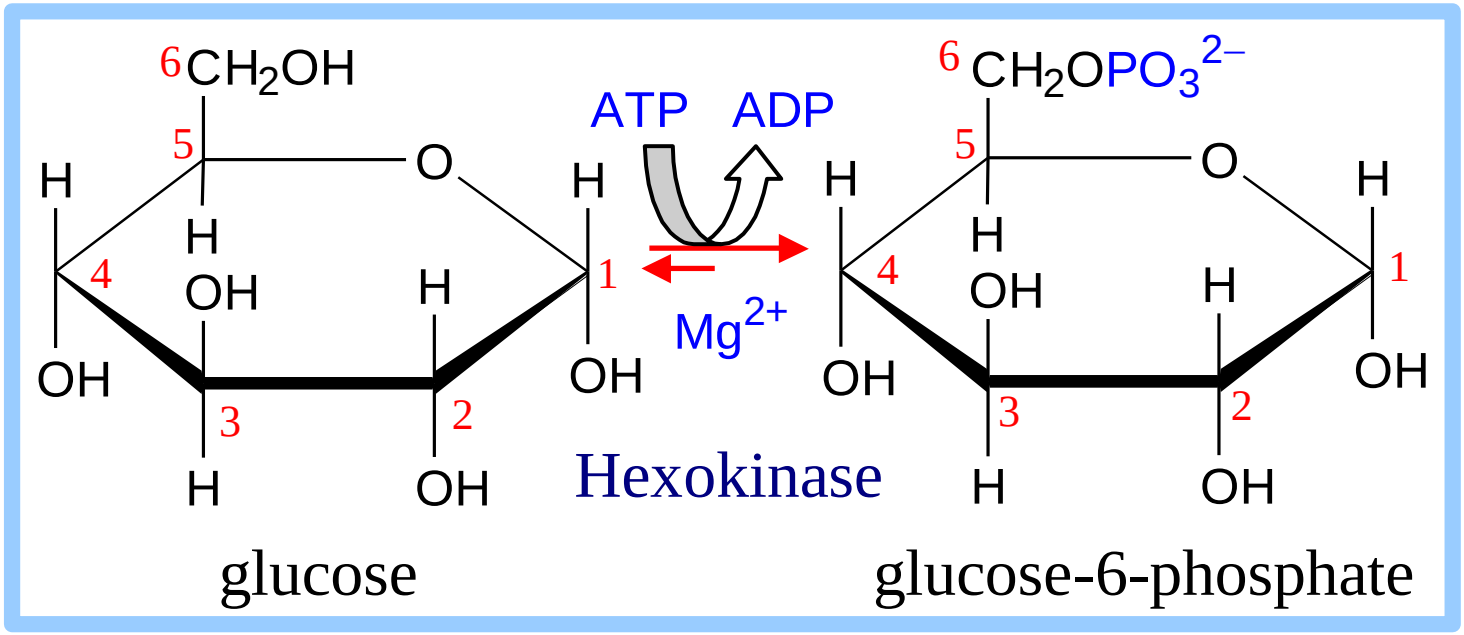
Glucose + ATP → glucose-6-P + ADP

The reaction involves nucleophilic attack of the C6 hydroxyl O of glucose on P of the terminal phosphate of ATP.

ATP binds to the enzyme as a complex with **Mg^{++}**.



Mg^{++} interacts with negatively charged phosphate oxygen atoms, providing charge compensation & promoting a favorable conformation of ATP at the active site of the Hexokinase enzyme.



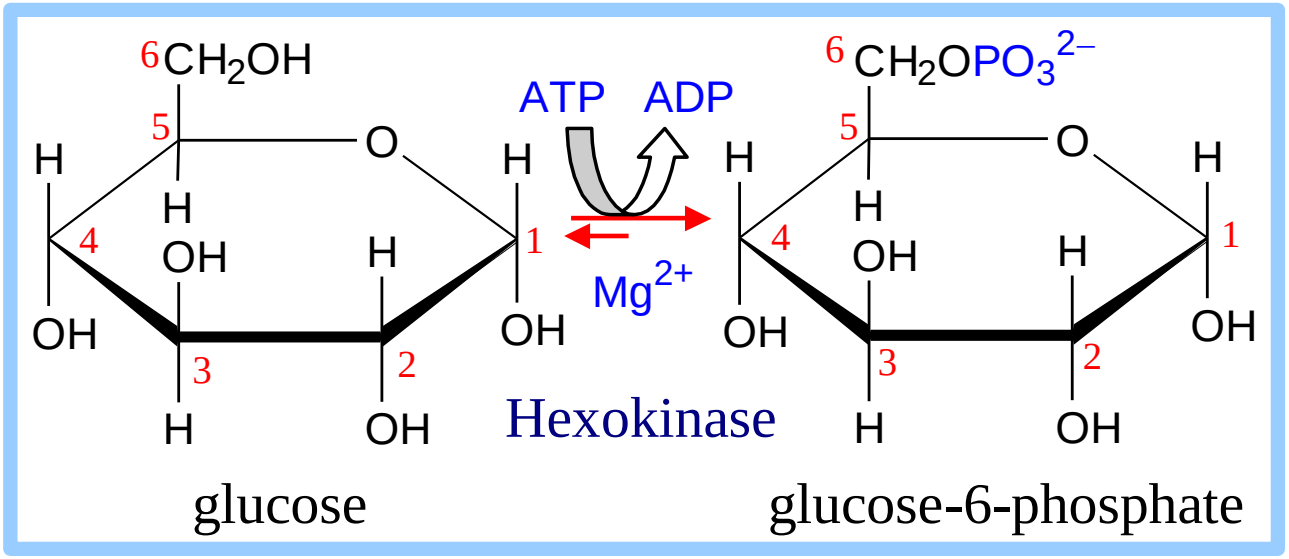
The reaction catalyzed by Hexokinase is highly **spontaneous**.

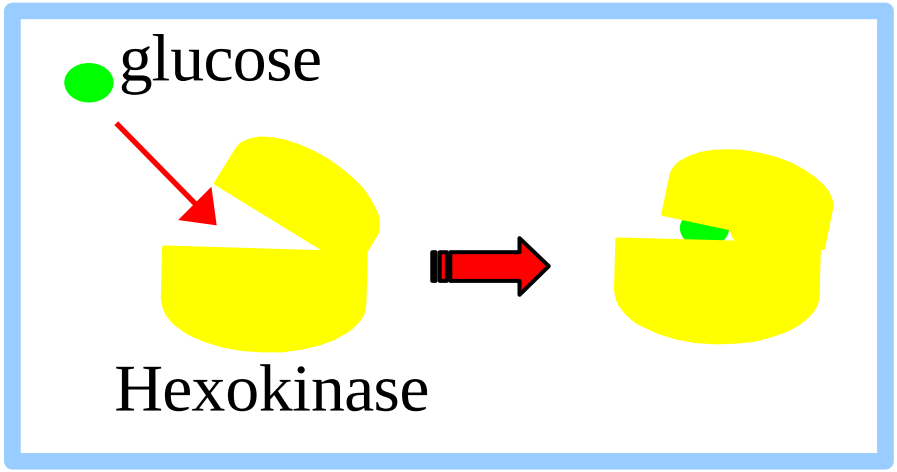
A phosphoanhydride bond of ATP (**~P**) is cleaved.

The phosphate ester formed in glucose-6-phosphate has a lower ΔG of hydrolysis.

Induced fit:

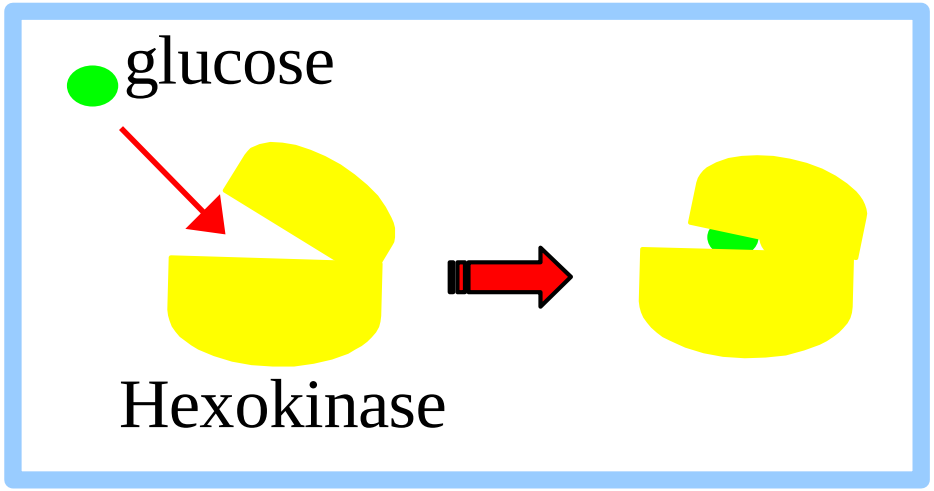
Glucose binding to Hexokinase stabilizes a **conformation** in which:





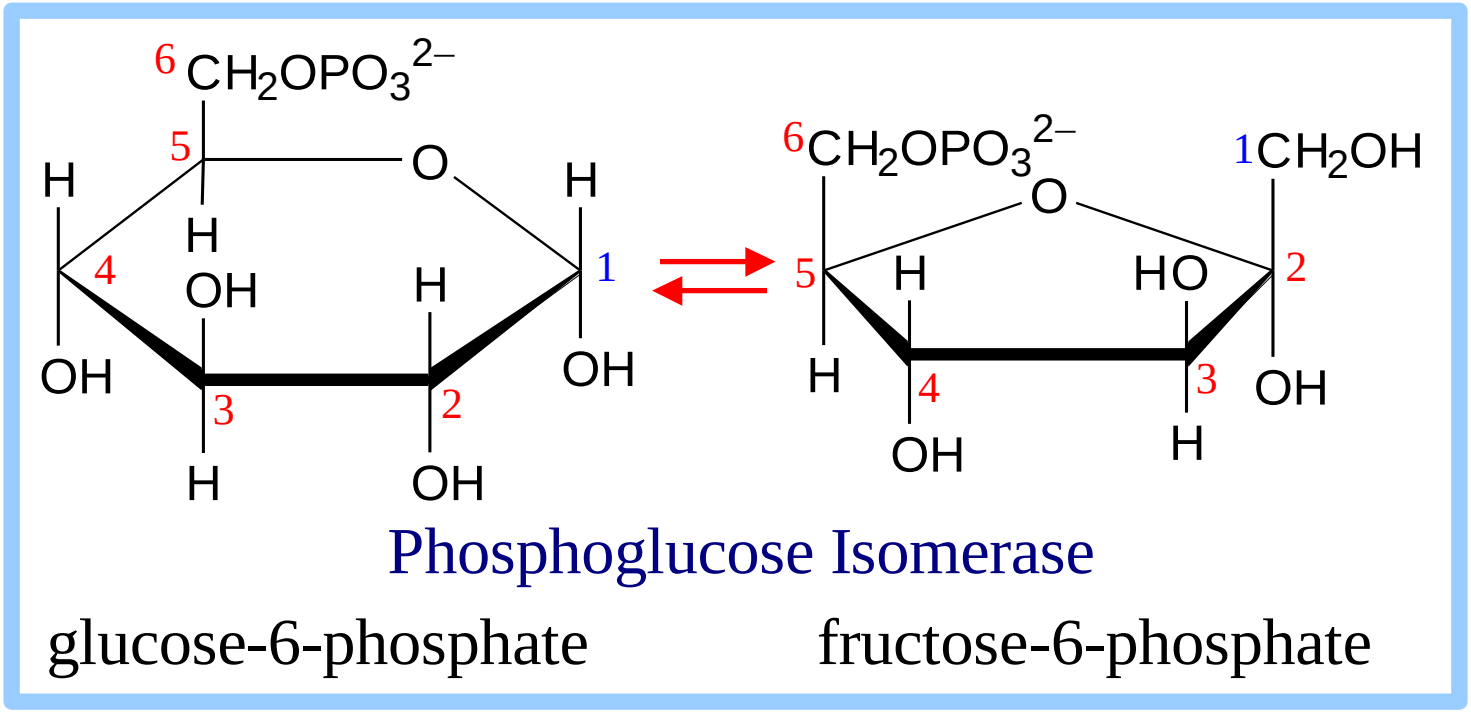
- the **C6 hydroxyl** of the bound glucose is **close to** the terminal **phosphate** of ATP, promoting catalysis.
- **water is excluded** from the active site.

  This prevents the enzyme from catalyzing ATP hydrolysis, rather than transfer of phosphate to glucose.



It is a **common motif** for an enzyme active site to be located at an interface between protein domains that are connected by a flexible hinge region.

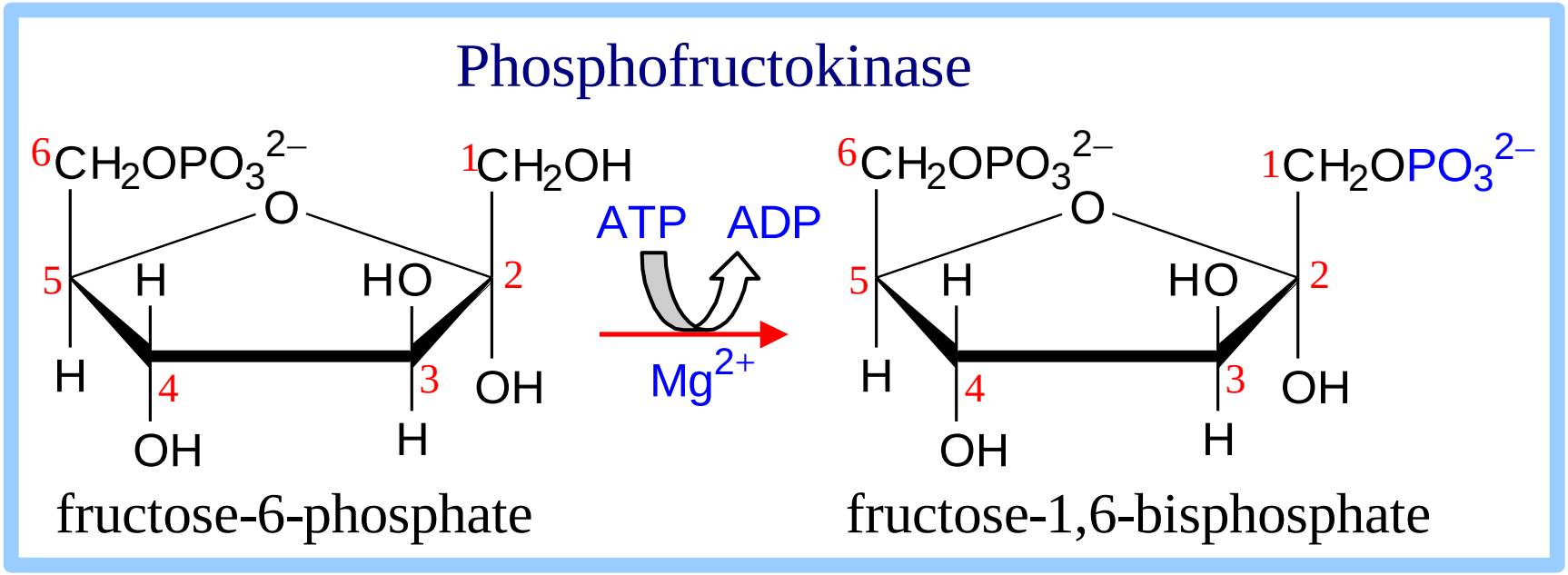
The **structural flexibility** allows access to the active site, while permitting precise positioning of active site residues, and in some cases exclusion of water, as substrate binding promotes a particular conformation.



2. **Phosphoglucose Isomerase** catalyzes:

glucose-6-P (aldose) ←→ **fructose-6-P** (ketose)

The mechanism involves acid/base catalysis, with ring opening, isomerization via an **enediolate intermediate**, and then ring closure. A similar reaction catalyzed by Triosephosphate Isomerase will be presented in detail.



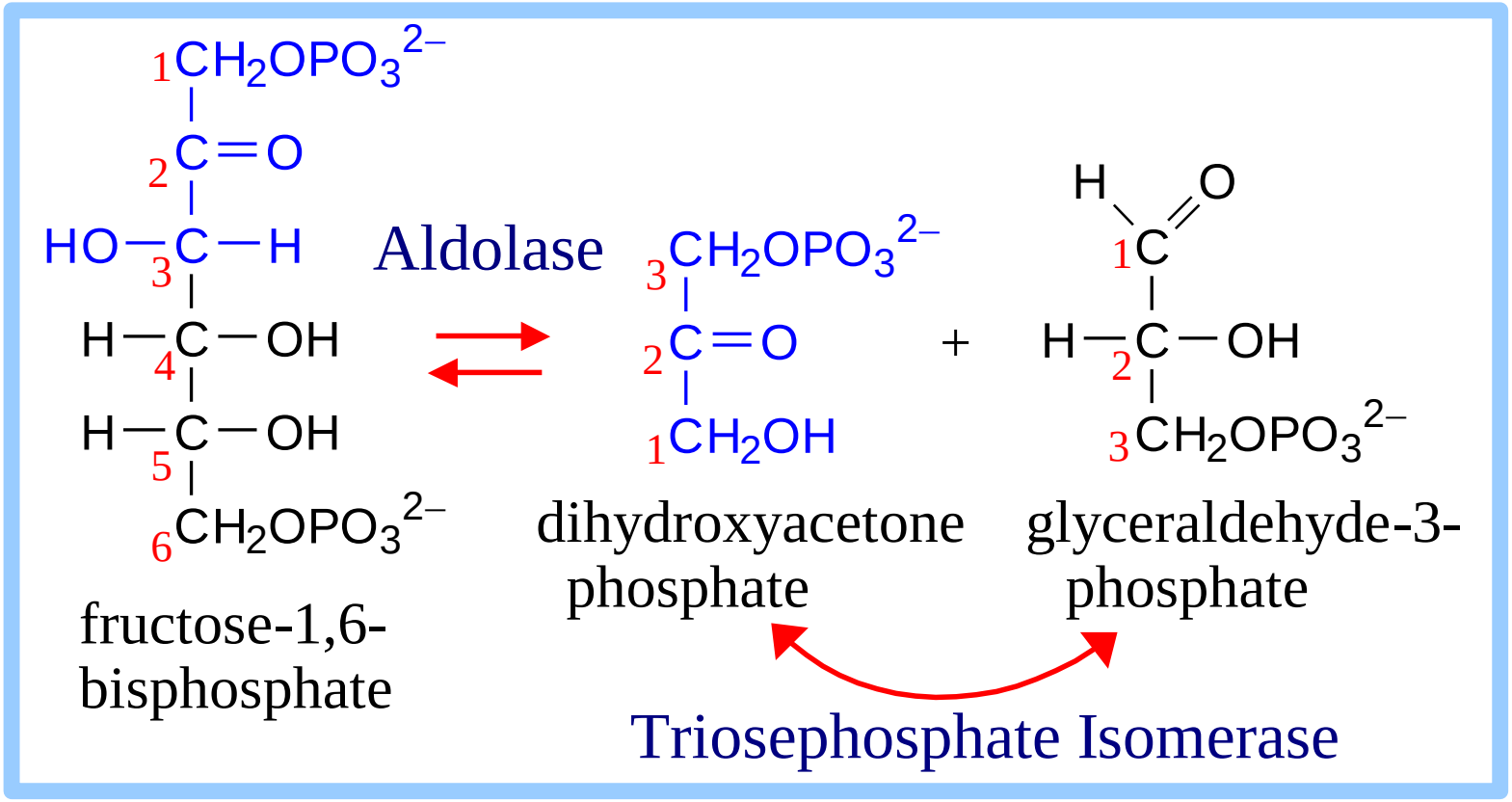
3. Phosphofructokinase catalyzes:

fructose-6-P + ATP → fructose-1,6-bisP + ADP

This highly **spontaneous** reaction has a mechanism similar to that of Hexokinase.

The Phosphofructokinase reaction is the **rate-limiting step** of Glycolysis.

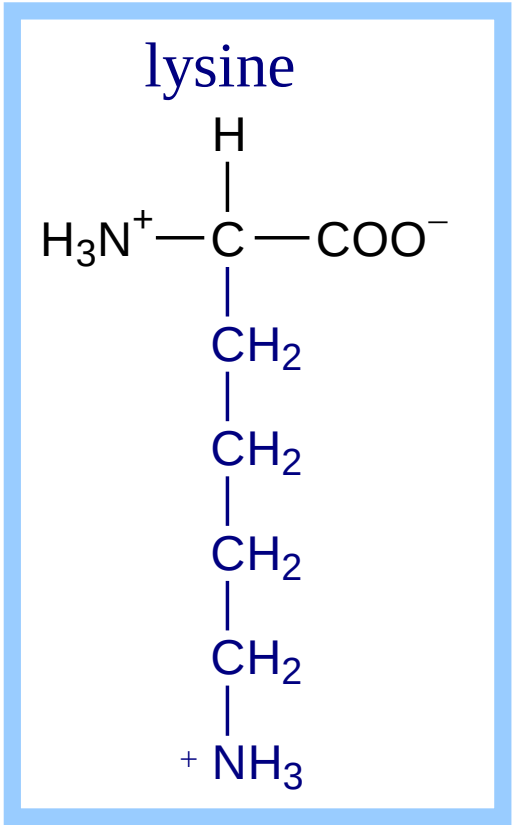
The enzyme is highly **regulated**, as will be discussed later.

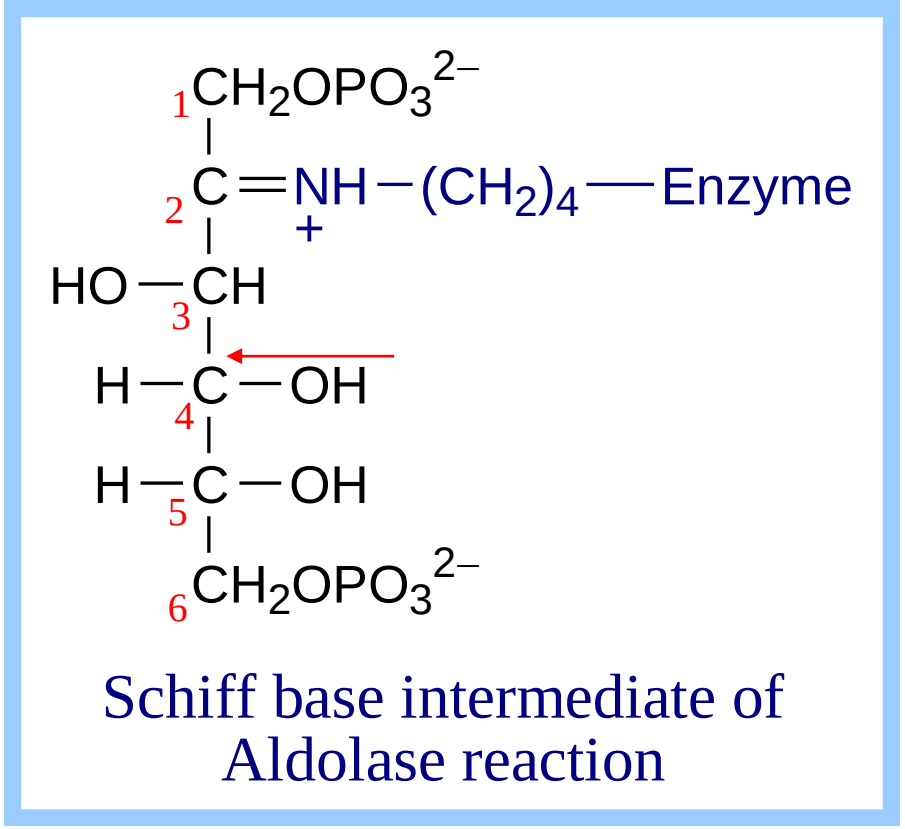


4. Aldolase catalyzes: **fructose-1,6-bisphosphate ←→ dihydroxyacetone-P + glyceraldehyde-3-P**

The reaction is an **aldol cleavage**, the reverse of an aldol condensation.

Note that C atoms are renumbered in products of Aldolase.

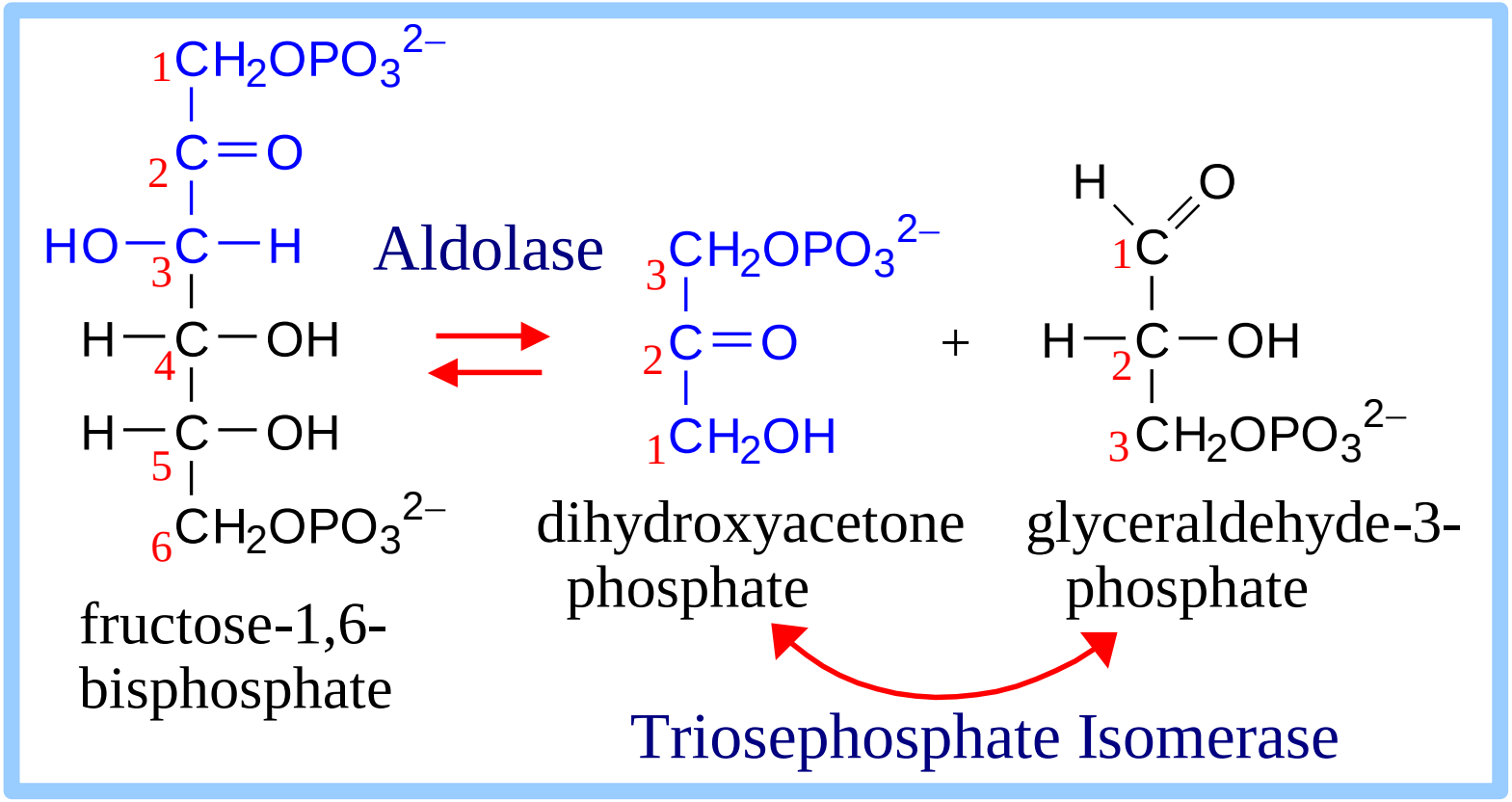




A **lysine** residue at the active site functions in catalysis.

The **keto** group of fructose-1,6-bisphosphate reacts with the ε-amino group of the active site lysine, to form a protonated **Schiff base** intermediate.

Cleavage of the bond between C3 & C4 follows.

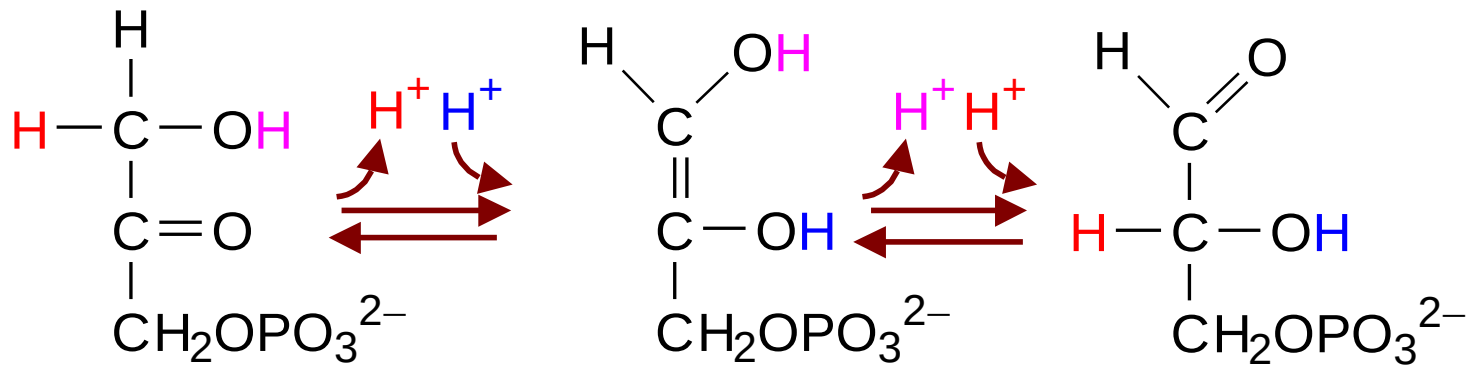


5. Triose Phosphate Isomerase (TIM) catalyzes:

dihydroxyacetone-P ←→ glyceraldehyde-3-P

Glycolysis continues from glyceraldehyde-3-P. TIM's K_{eq} favors dihydroxyacetone-P. Removal of glyceraldehyde-3-P by a subsequent spontaneous reaction allows throughput.

Triosephosphate Isomerase



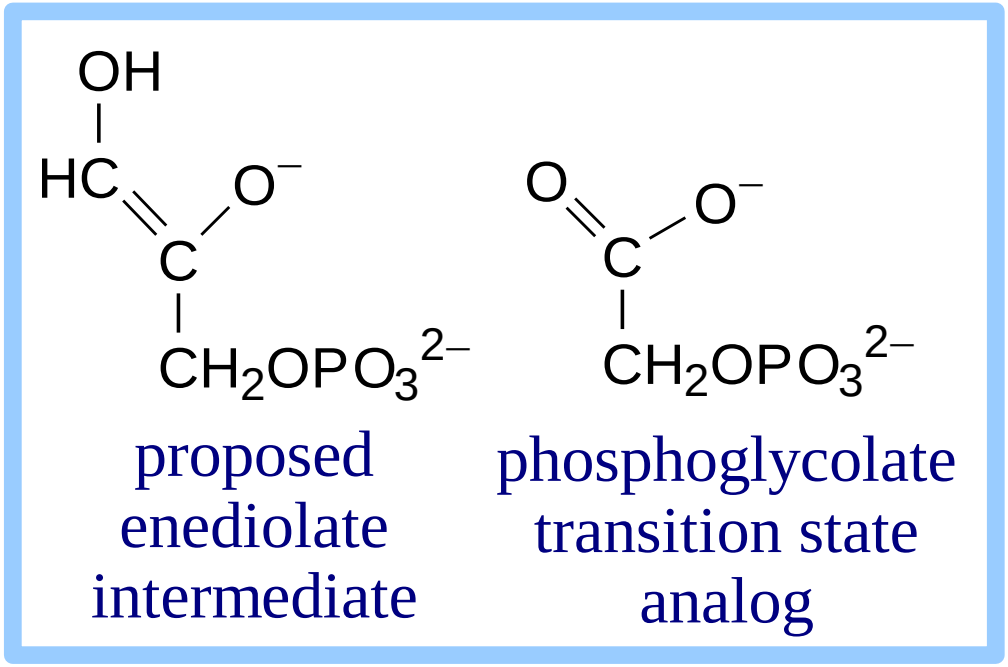
dihydroxyacetone phosphate

enediol intermediate

glyceraldehyde-3-phosphate

The ketose/aldose conversion involves **acid/base catalysis**, and is thought to proceed via an **enediol** intermediate, as with Phosphoglucose Isomerase.

Active site Glu and His residues are thought to extract and donate protons during catalysis.



2-Phosphoglycolate is a **transition state analog** that binds tightly at the active site of Triose Phosphate Isomerase (TIM).

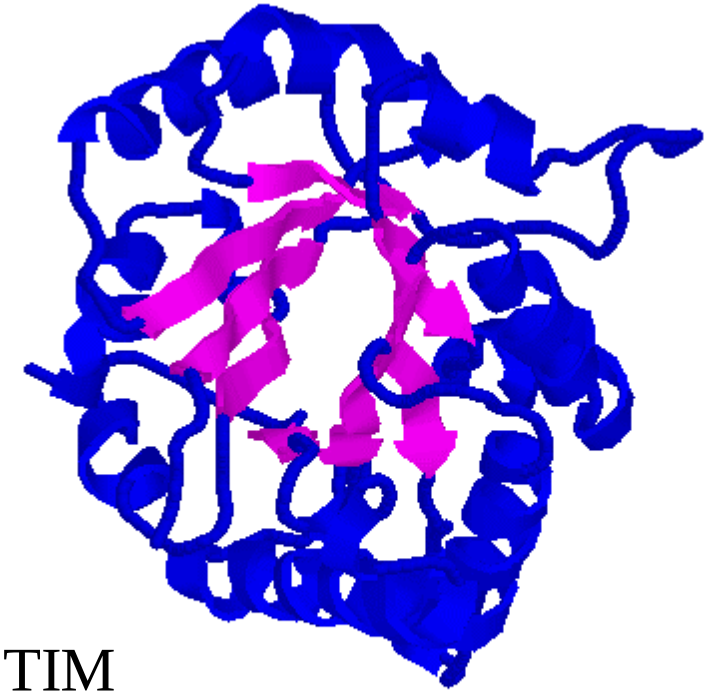
This inhibitor of catalysis by TIM is similar in structure to the proposed enediolate intermediate.

TIM is judged a "perfect enzyme." Reaction rate is limited only by the rate that substrate collides with the enzyme.

Triosephosphate Isomerase structure is an **αβ barrel**, or TIM barrel.

In an αβ barrel there are 8 parallel β-strands surrounded by 8 α-helices.

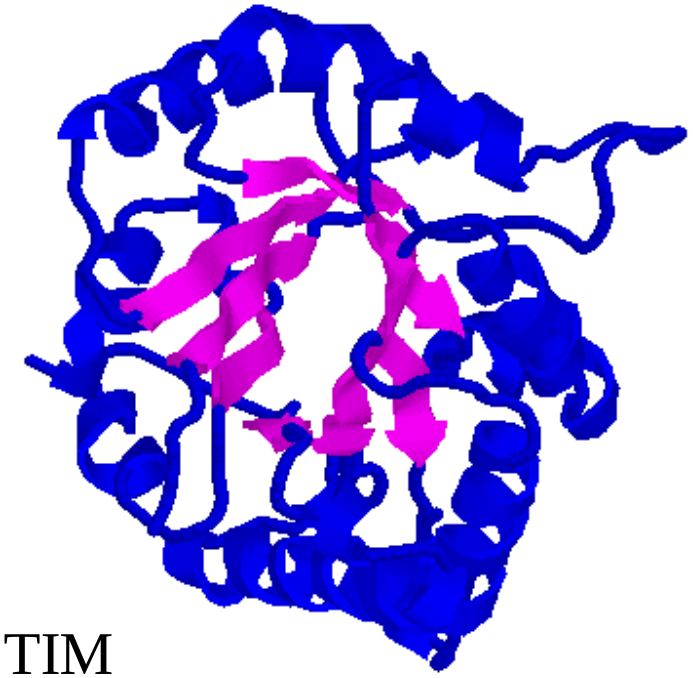
Short loops connect alternating β-strands & α-helices.



TIM

TIM barrels serve as scaffolds for active site residues in a diverse array of enzymes.

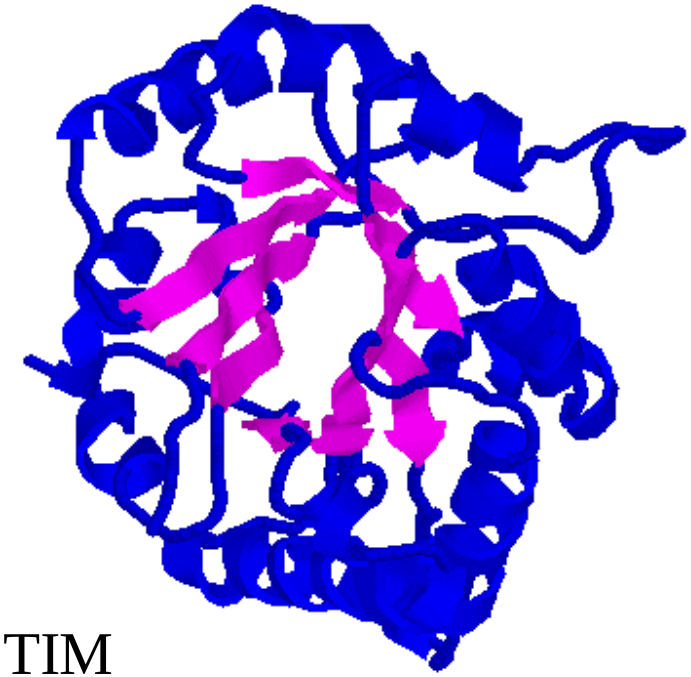
Residues of the **active site** are always at the same end of the barrel, on C-terminal ends of β-strands & loops connecting these to α-helices.



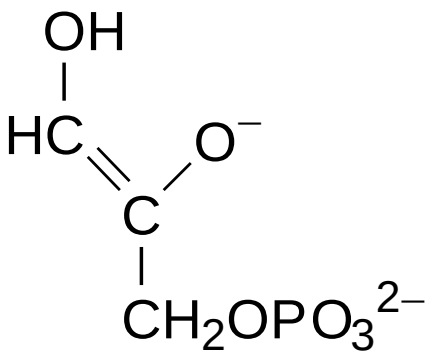
TIM

There is debate whether the many different enzymes with TIM barrel structures are evolutionarily related.

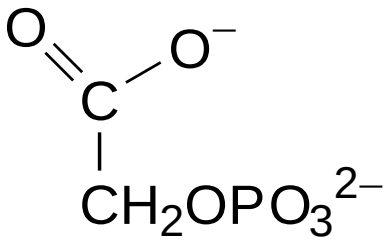
In spite of the structural similarities there is tremendous **diversity in catalytic functions** of these enzymes and little sequence homology.



TIM



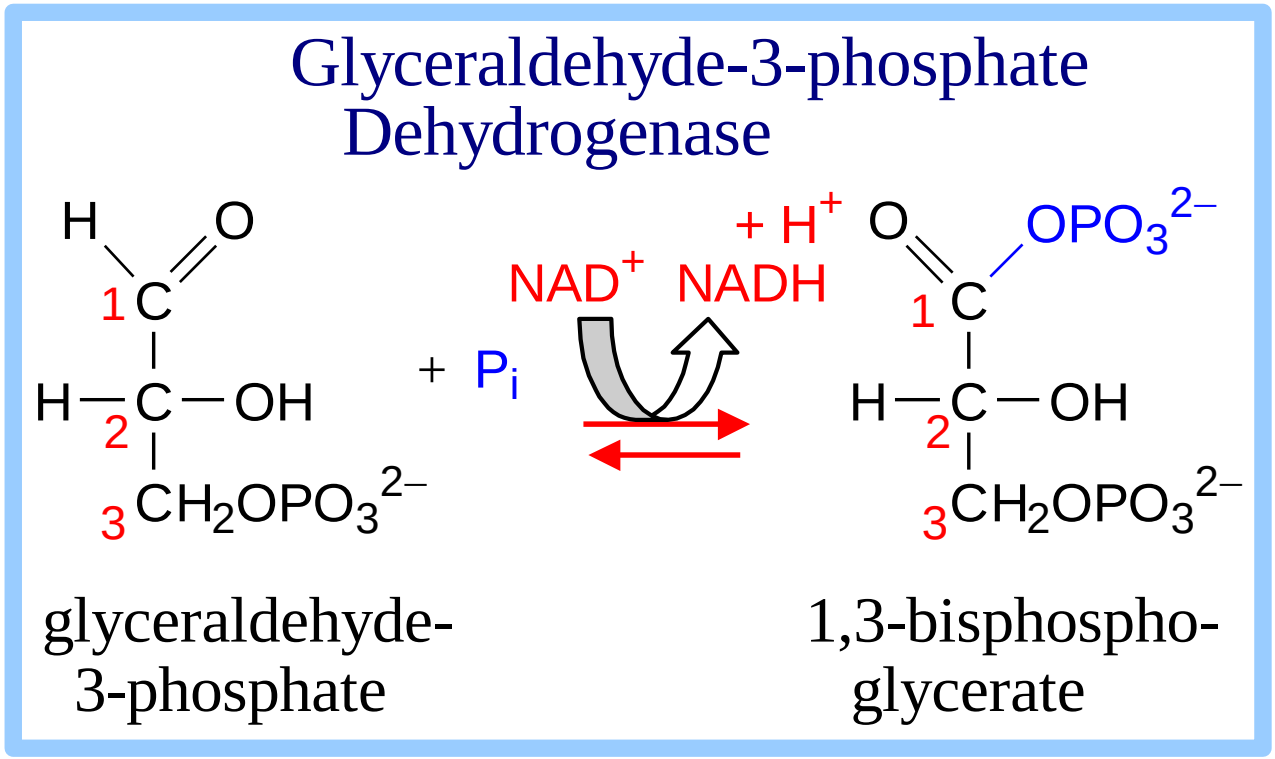
proposed enediolate intermediate



phosphoglycolate transition state analog

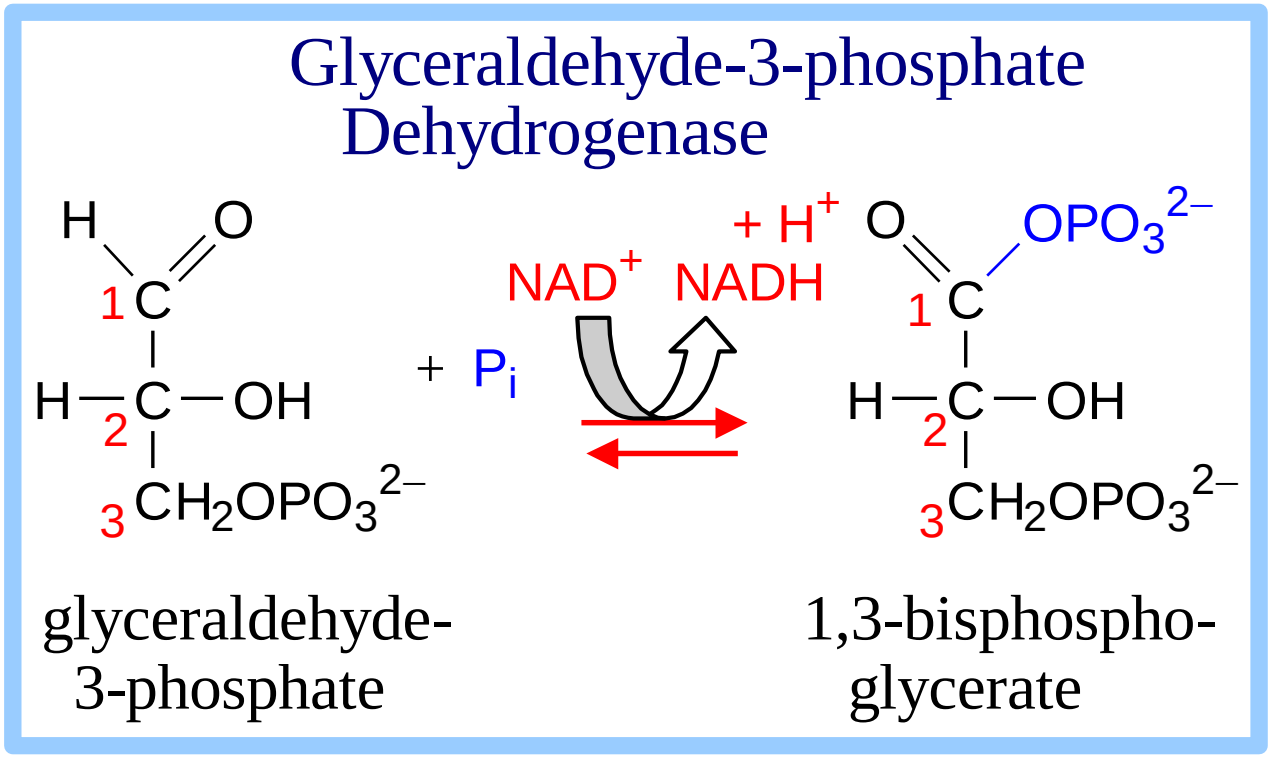
Explore the structure of the Triosephosphate Isomerase (TIM) homodimer, with the transition state inhibitor 2-phosphoglycolate bound to one of the TIM monomers.

Note the structure of the TIM barrel, and the loop that forms a lid that closes over the active site after binding of the substrate.



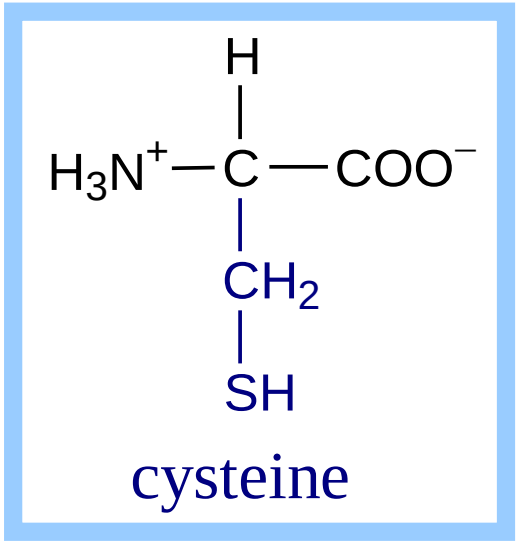
6. Glyceraldehyde-3-phosphate Dehydrogenase catalyzes:

glyceraldehyde-3-P + NAD^+ + P_i ←→ 1,3-bisphosphoglycerate + NADH + H^+

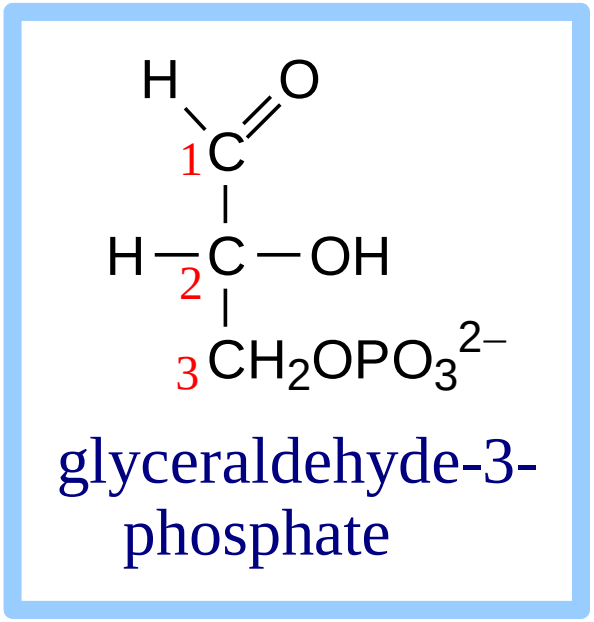


Exergonic oxidation of the aldehyde in glyceraldehyde-3-phosphate, to a carboxylic acid, drives formation of an **acyl phosphate**, a "high energy" bond (**~P**).

This is the only step in Glycolysis in which **NAD^+** is reduced to NADH.



cysteine

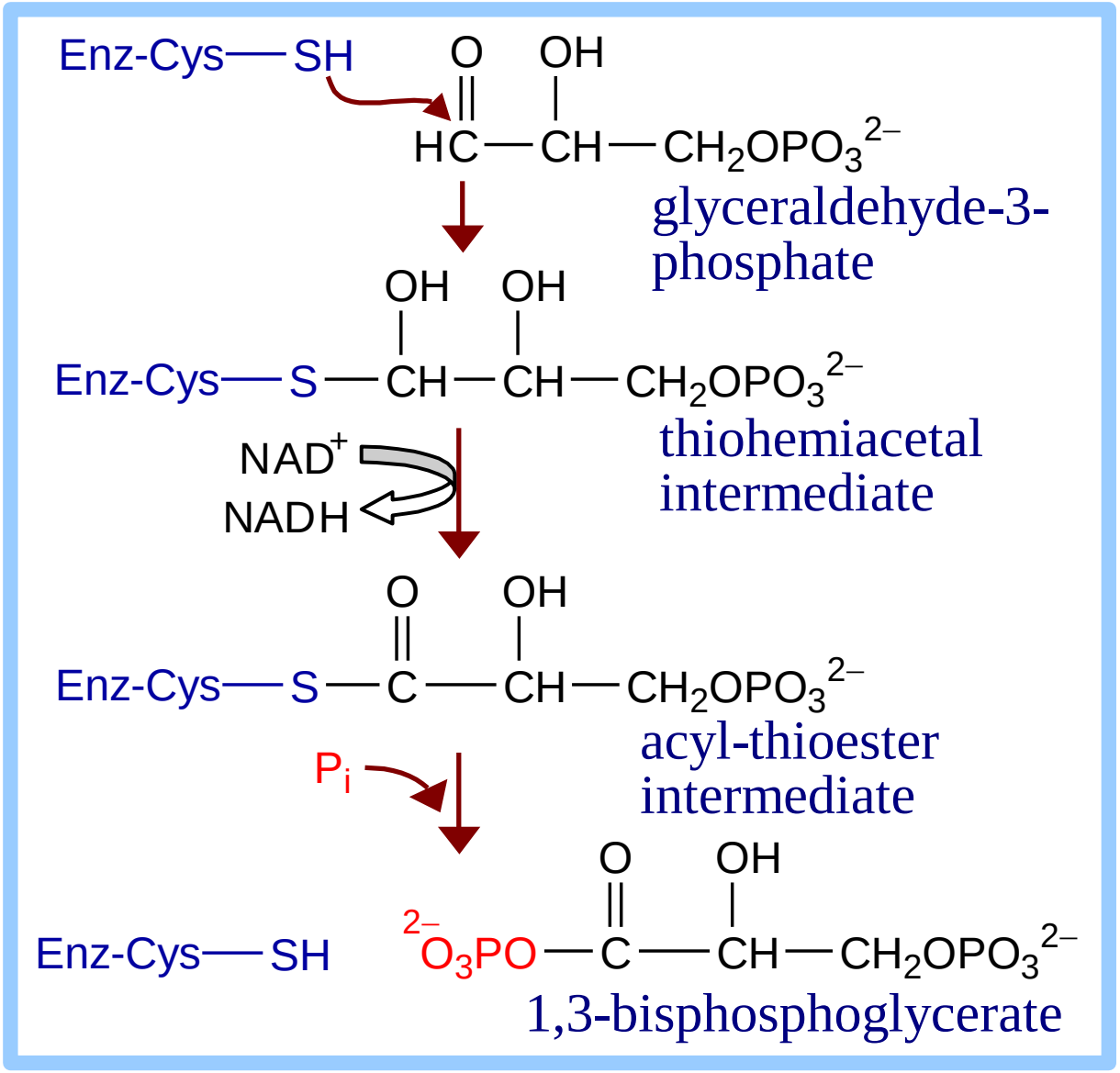


glyceraldehyde-3-phosphate

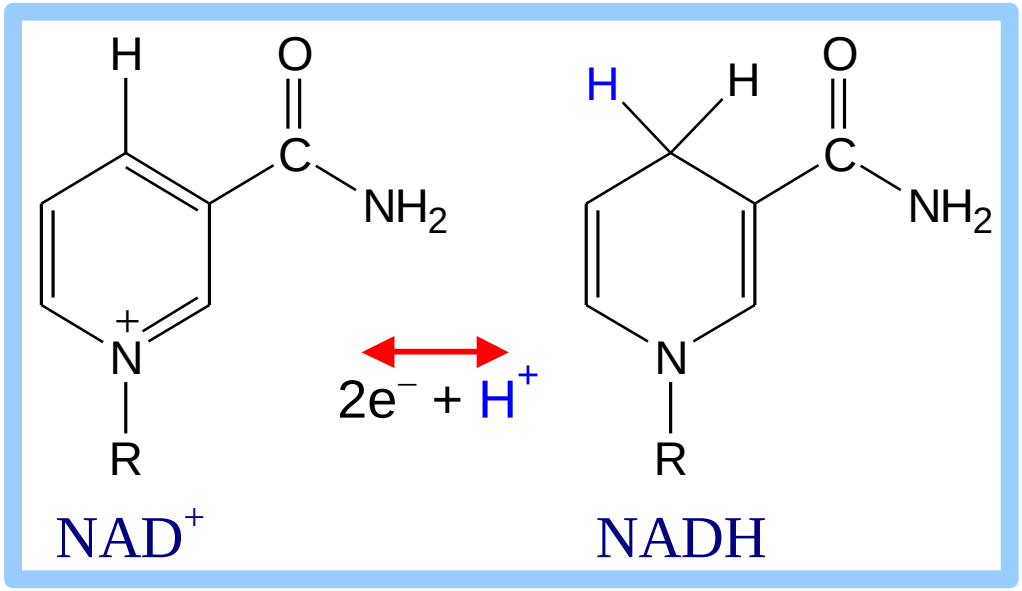
A **cysteine thiol** at the active site of Glyceraldehyde-3-phosphate Dehydrogenase has a role in catalysis.

The aldehyde of glyceraldehyde-3-phosphate reacts with the cysteine thiol to form a **thiohemiacetal** intermediate.

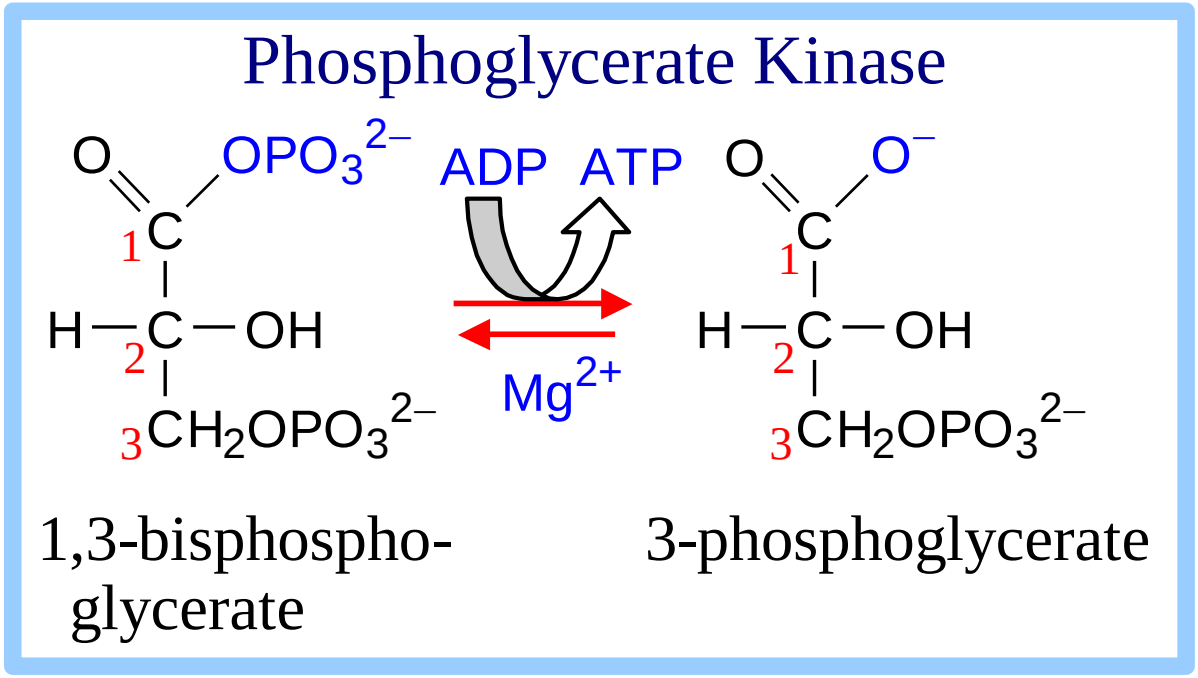
Oxidation to a carboxylic acid (in a ~ **thioester**) occurs, as NAD^+ is reduced to **NADH**.



The "high energy" acyl thioester is attacked by P_i to yield the acyl phosphate (**~P**) product.



Recall that NAD^+ accepts 2 e^- plus one H^+ (a hydride) in going to its reduced form.



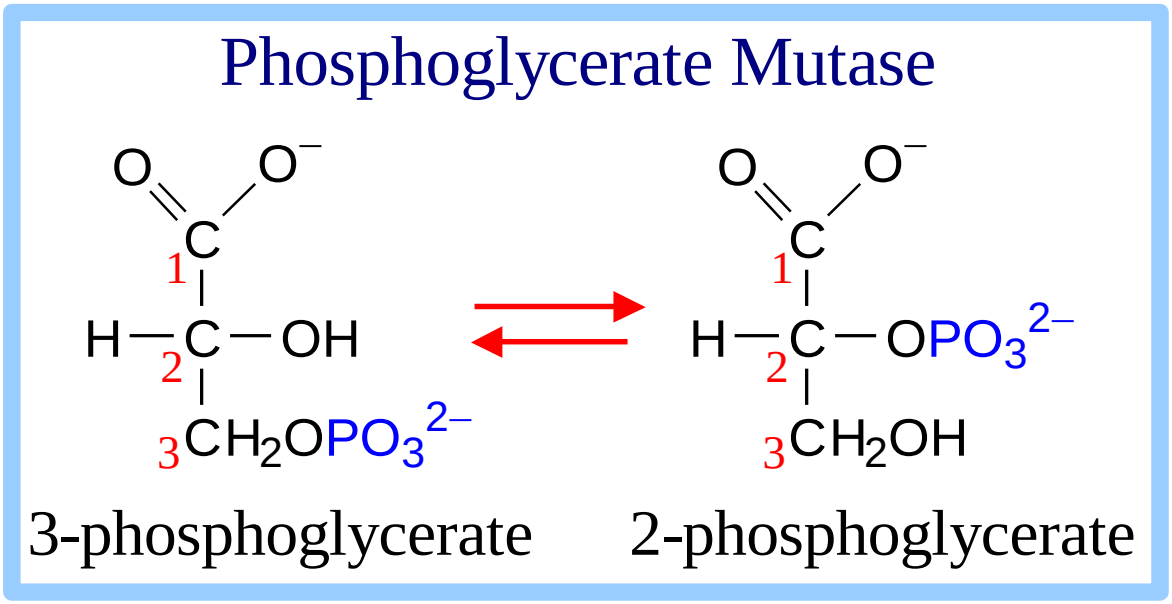
7. Phosphoglycerate Kinase catalyzes:

1,3-bisphosphoglycerate + ADP ←→

3-phosphoglycerate + ATP

This phosphate transfer is reversible (low ΔG), since one **~P** bond is cleaved & another synthesized.

The enzyme undergoes substrate-induced conformational change similar to that of Hexokinase.

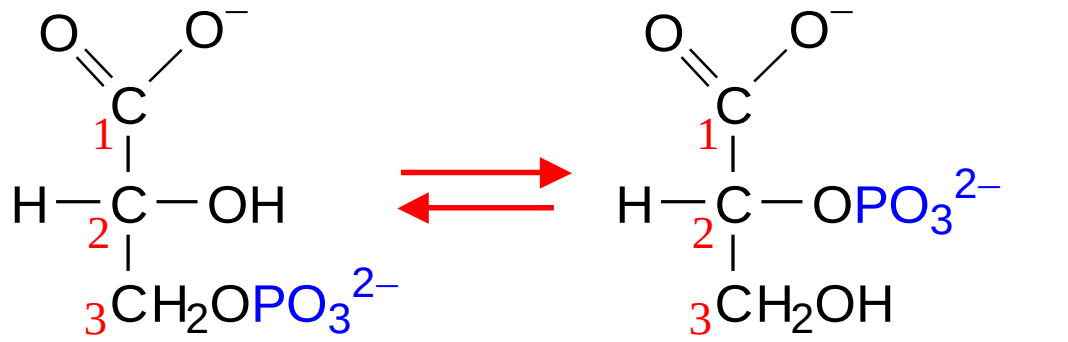


8. Phosphoglycerate Mutase catalyzes:

3-phosphoglycerate ←→ 2-phosphoglycerate

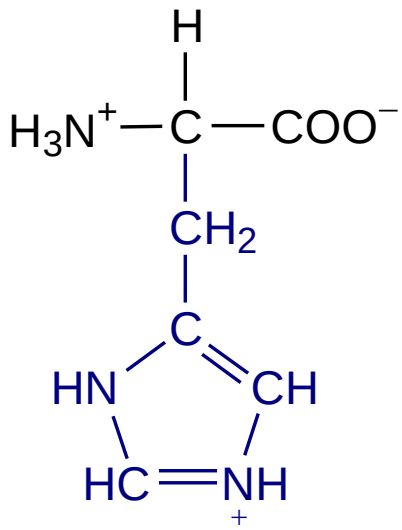
Phosphate is shifted from the OH on C3 to the OH on C2.

Phosphoglycerate Mutase



3-phosphoglycerate 2-phosphoglycerate

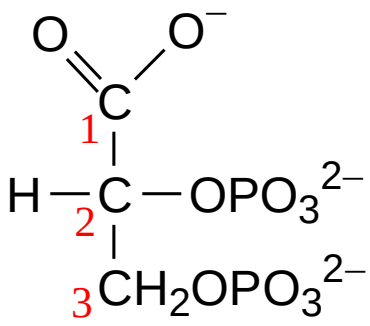
histidine



An active site **histidine** side-chain participates in P_i transfer, by donating & accepting phosphate.

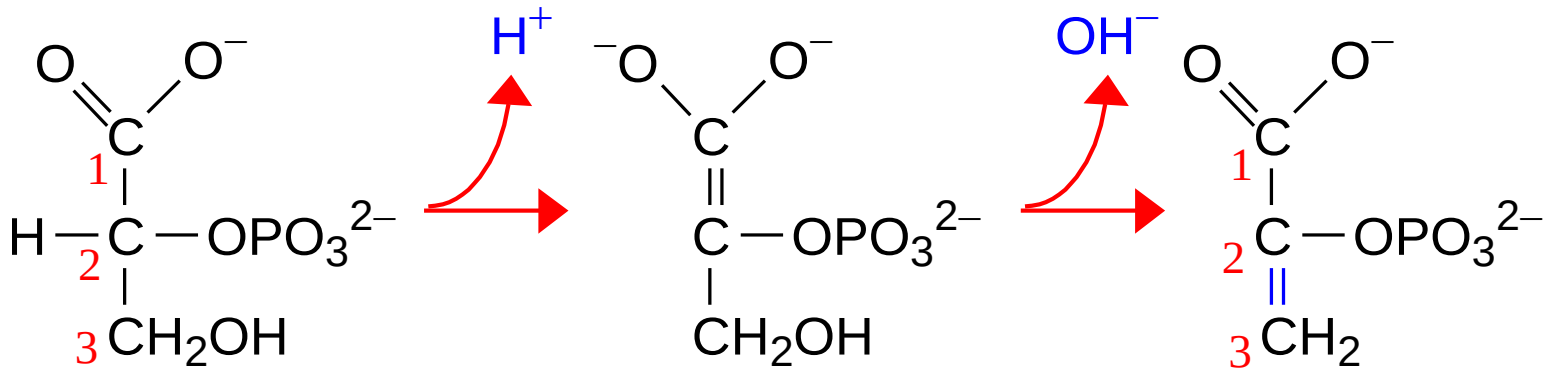
The process involves a **2,3-bisphosphate** intermediate.

View an animation of the Phosphoglycerate Mutase reaction.



2,3-bisphosphoglycerate

Enolase



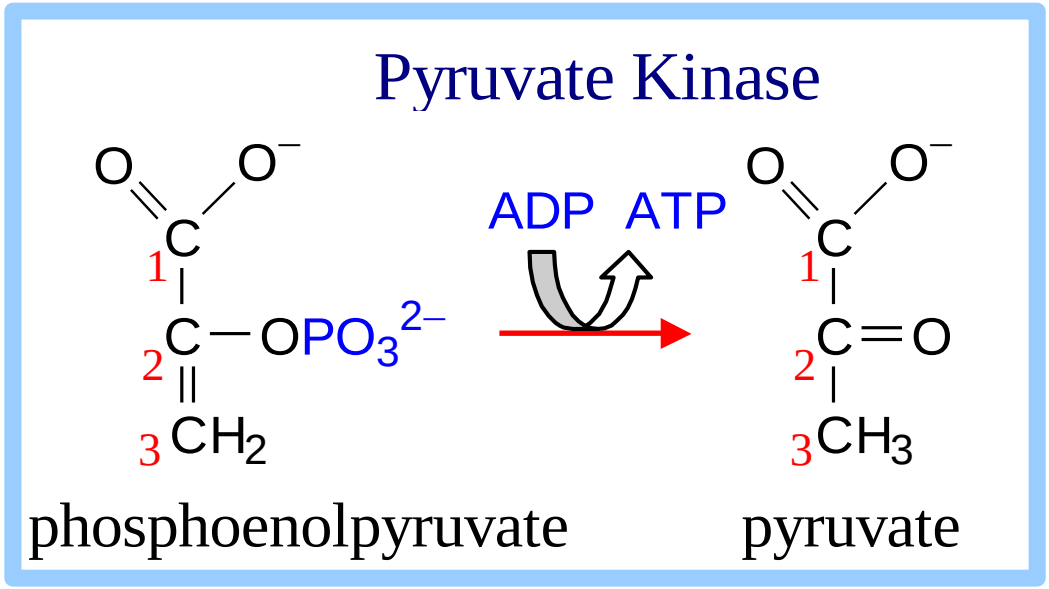
9. Enolase catalyzes:

2-phosphoglycerate ←→ phosphoenolpyruvate + H_2O

This dehydration reaction is **Mg^{++}-dependent**.

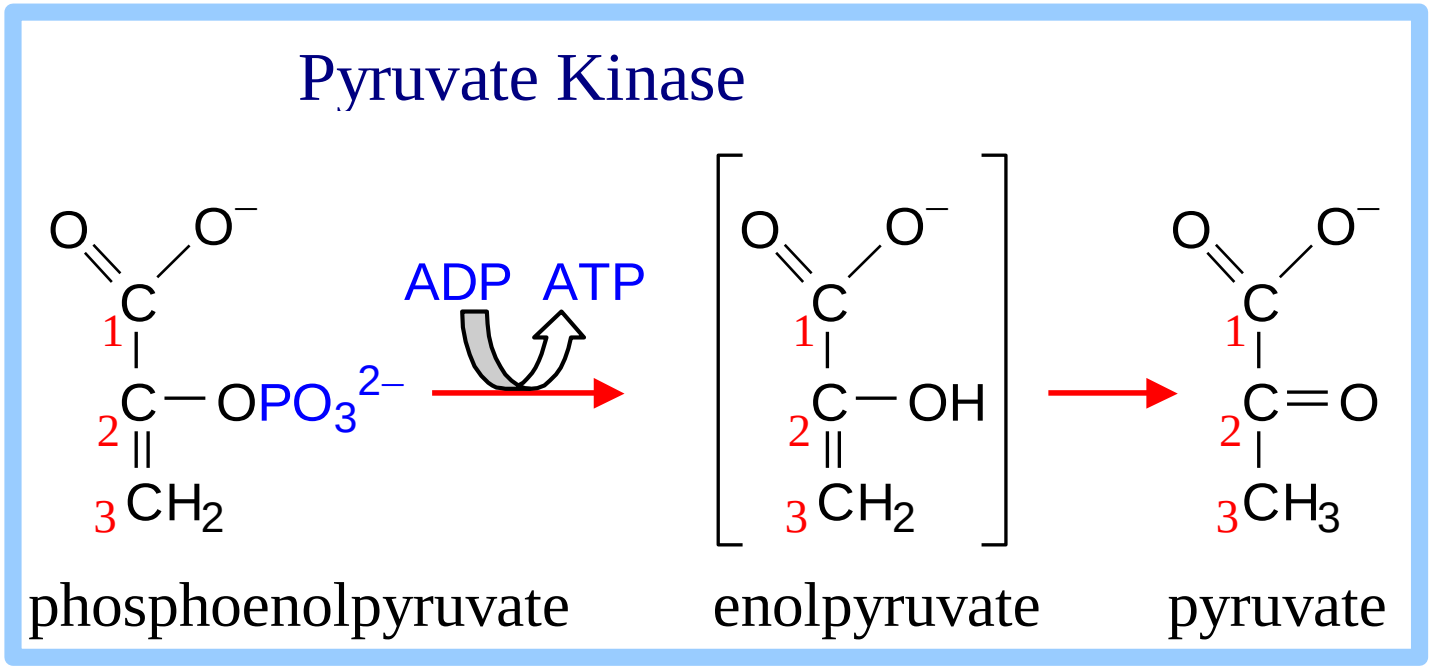
2 Mg^{++} ions interact with oxygen atoms of the substrate **carboxyl** group at the active site.

The Mg^{++} ions help to stabilize the enolate anion intermediate that forms when a Lys extracts H^+ from C #2.



10. Pyruvate Kinase catalyzes:

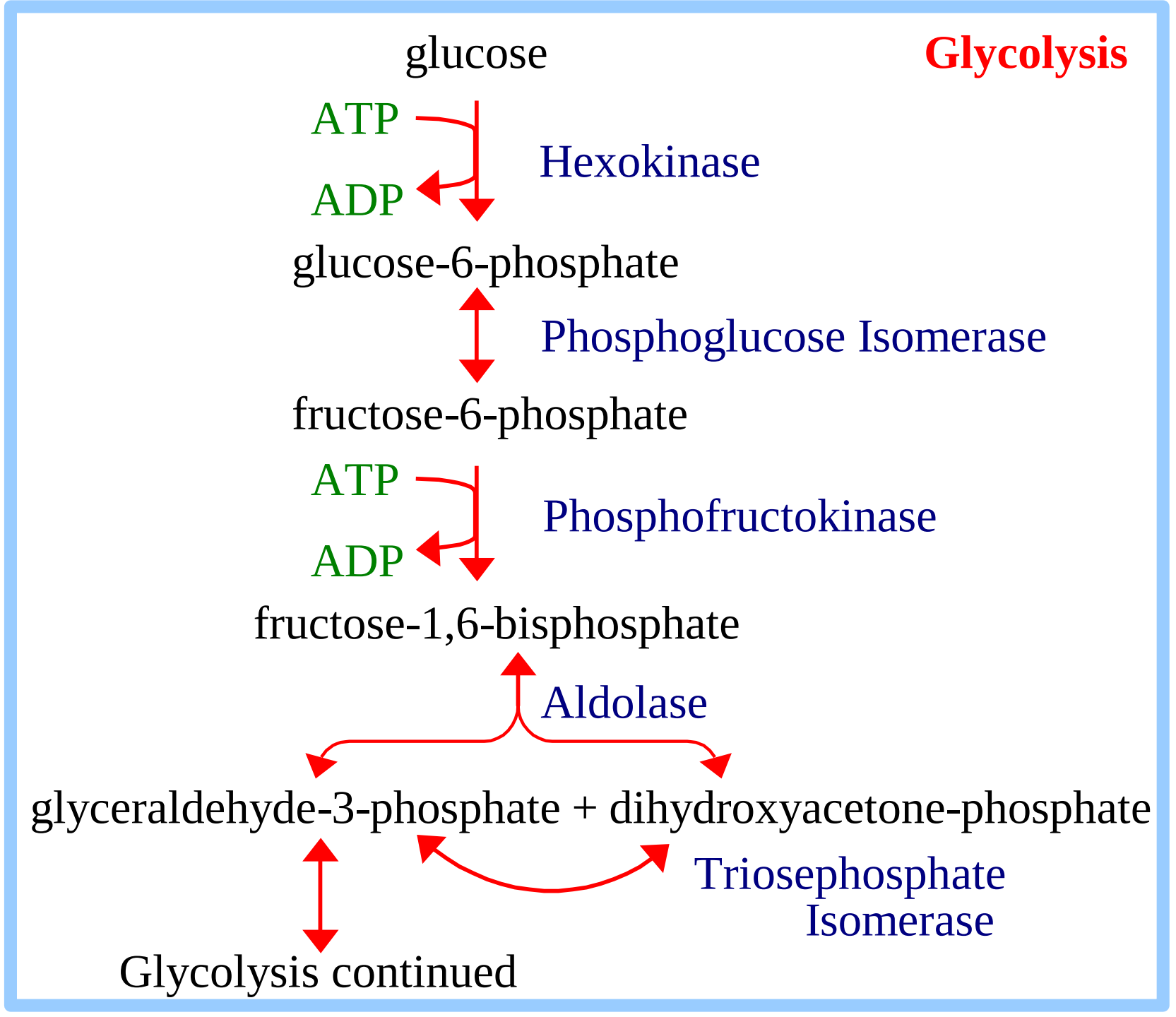
phosphoenolpyruvate + ADP → pyruvate + ATP



This phosphate transfer from PEP to ADP is **spontaneous**.

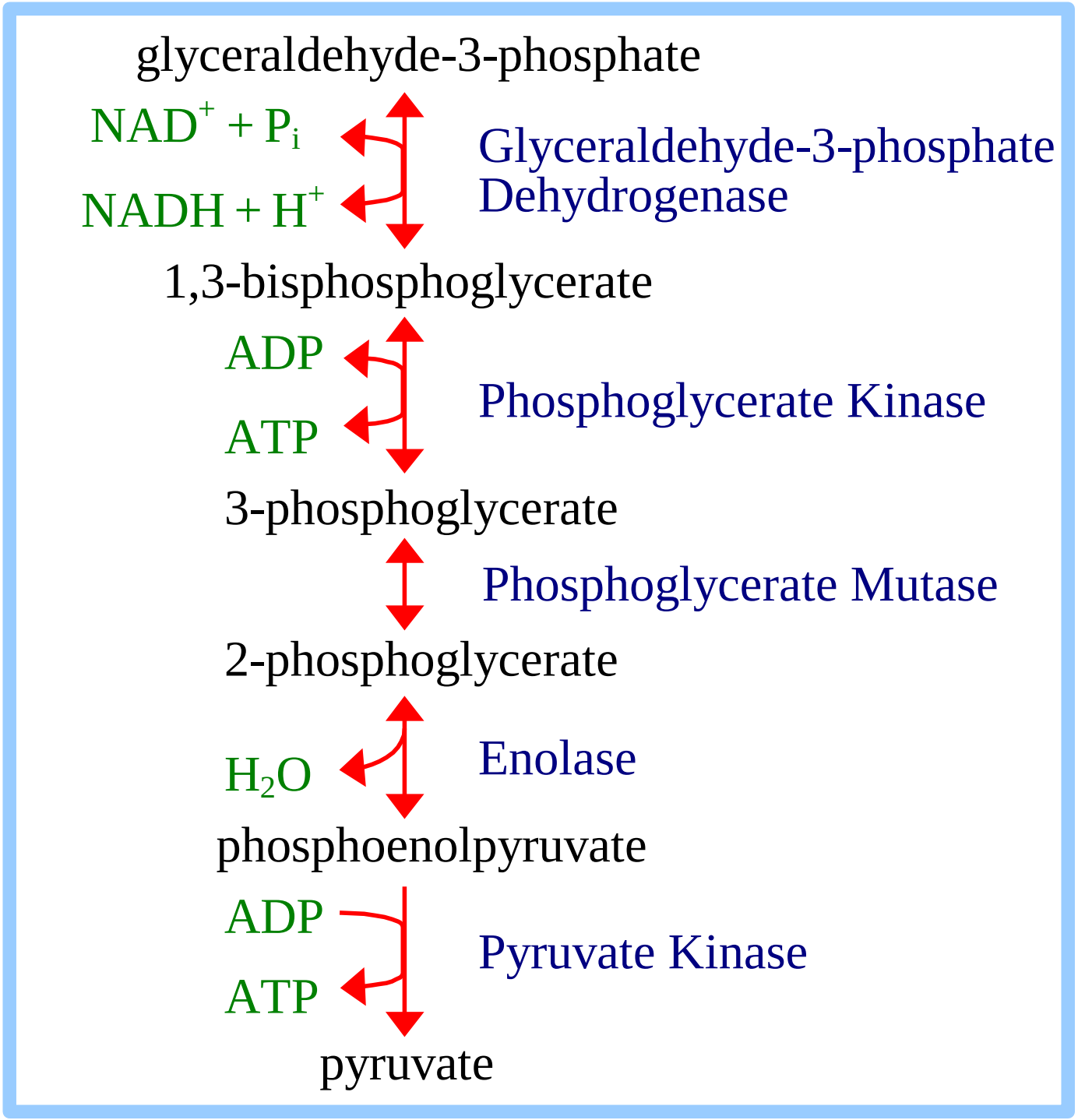
- PEP has a larger ΔG of phosphate hydrolysis than ATP.
- Removal of P_i from PEP yields an unstable enol, which spontaneously converts to the keto form of pyruvate.

Required inorganic **cations** K^+ and Mg^{++} bind to anionic residues at the active site of Pyruvate Kinase.


Glycolysis
glucose
ATP
ADP
Hexokinase
glucose-6-phosphate
Phosphoglucose Isomerase
fructose-6-phosphate
ATP
ADP
Phosphofructokinase
fructose-1,6-bisphosphate
Aldolase
glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate
Triosephosphate
Isomerase
Glycolysis continued

Glycolysis continued.

Recall that there are 2 GAP per glucose.



Glycolysis

Balance sheet for **~P** bonds of ATP:

- How many ATP ~P bonds expended? **2**
- How many ~P bonds of ATP produced? (Remember there are two 3C fragments from glucose.) **4**
- Net production of **~P** bonds of ATP per glucose: **2**

Balance sheet for **~P** bonds of ATP:

- 2 ATP expended
- 4 ATP produced (2 from each of two 3C fragments from glucose)
- Net production of **2 ~P** bonds of **ATP** per glucose.

Glycolysis - total pathway, omitting H^+:

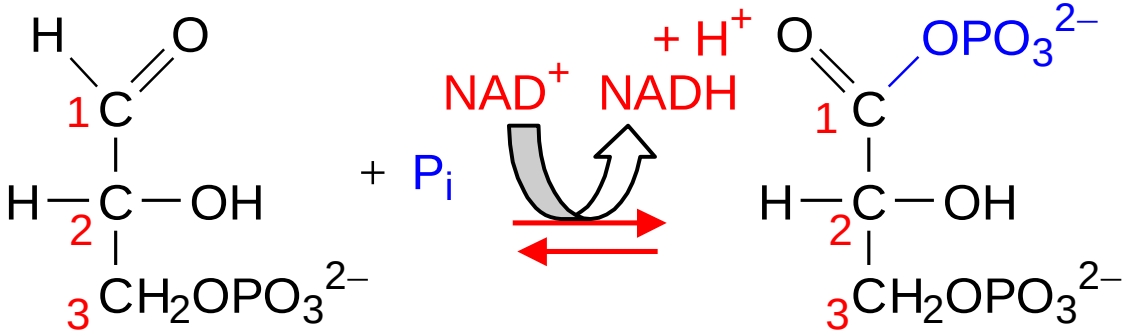
glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

In **aerobic organisms**:

- **pyruvate** produced in Glycolysis is oxidized to CO_2 via Krebs Cycle
- **NADH** produced in Glycolysis & Krebs Cycle is reoxidized via the respiratory chain, with production of much additional ATP.

Fermentation:

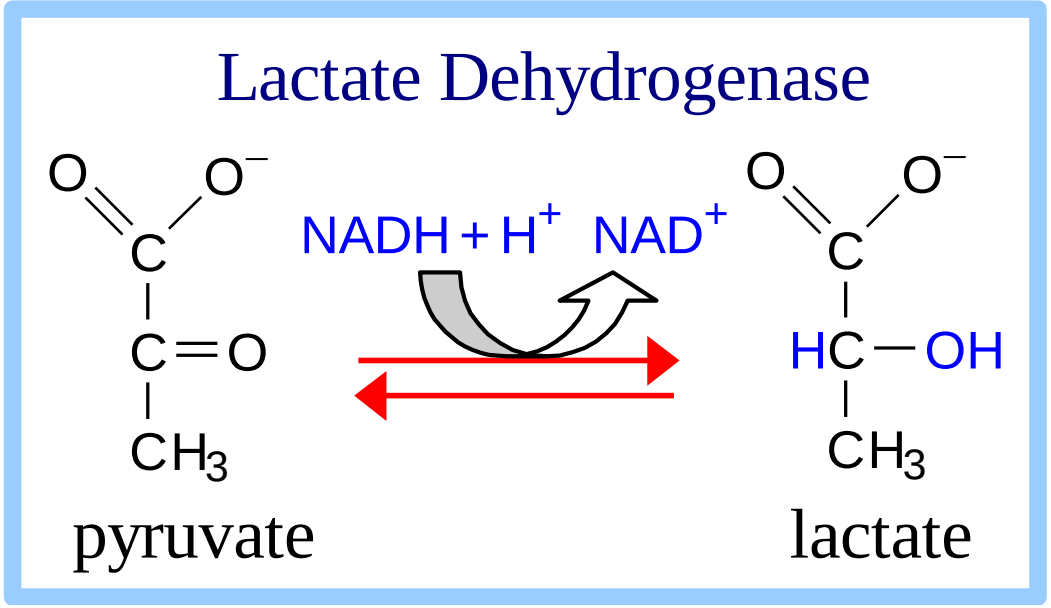
Anaerobic organisms lack a respiratory chain.



They **must reoxidize NADH** produced in Glycolysis through some other reaction, because **NAD^+** is needed for the Glyceraldehyde-3-phosphate Dehydrogenase reaction.

Usually NADH is reoxidized as **pyruvate** is converted to a **more reduced** compound.

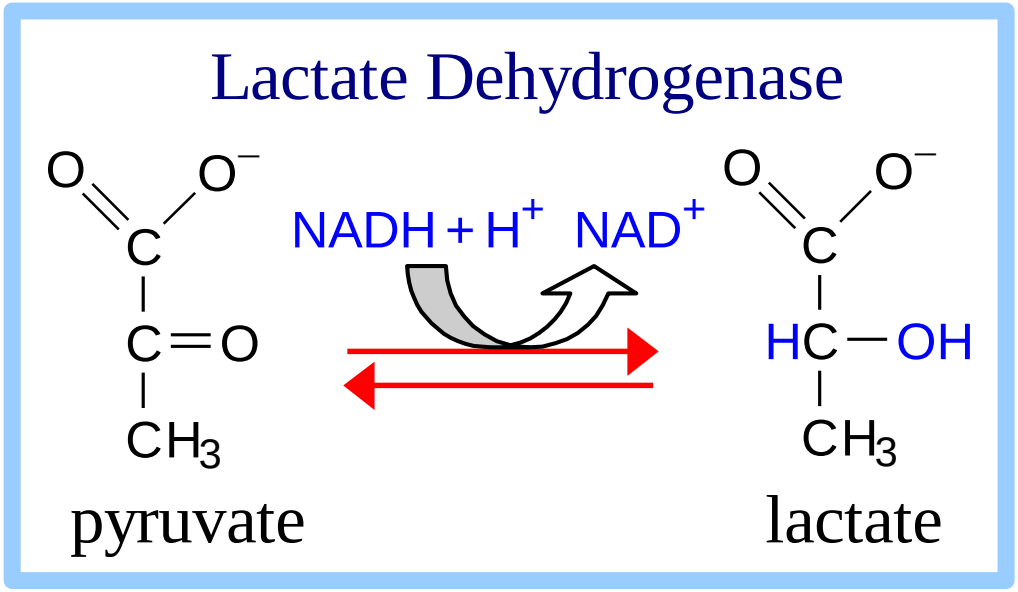
The complete pathway, including Glycolysis and the reoxidation of NADH, is called **fermentation**.



E.g., **Lactate Dehydrogenase** catalyzes **reduction** of the keto in **pyruvate** to a hydroxyl, yielding **lactate**, as NADH is oxidized to NAD^+.

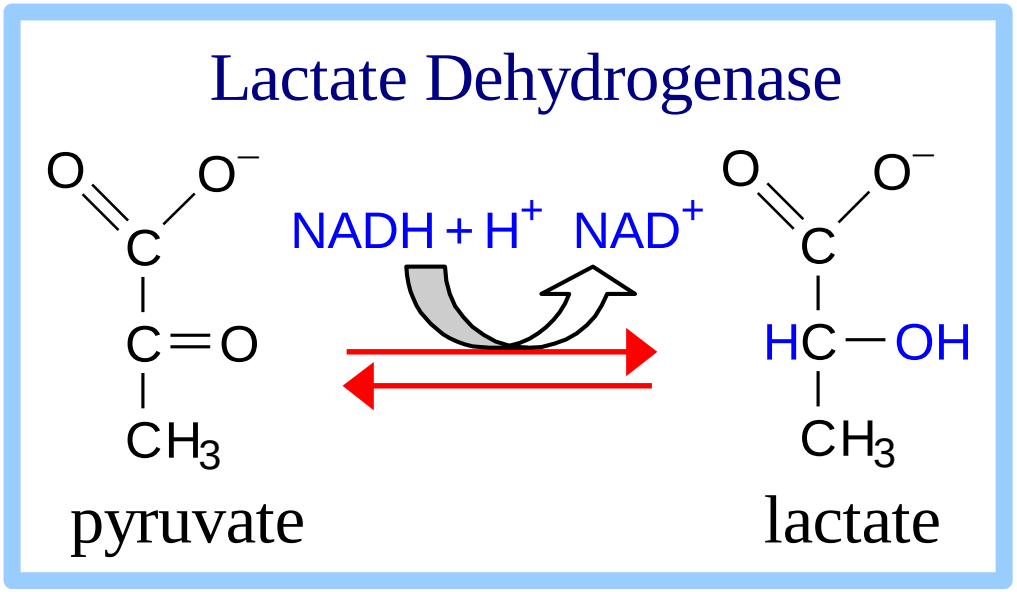
Lactate, in addition to being an end-product of fermentation, serves as a **mobile** form of **nutrient energy**, & possibly as a **signal** molecule in mammalian organisms.

Cell membranes contain **carrier** proteins that facilitate transport of lactate.



Skeletal muscles ferment glucose to **lactate** during exercise, when the exertion is brief and intense.

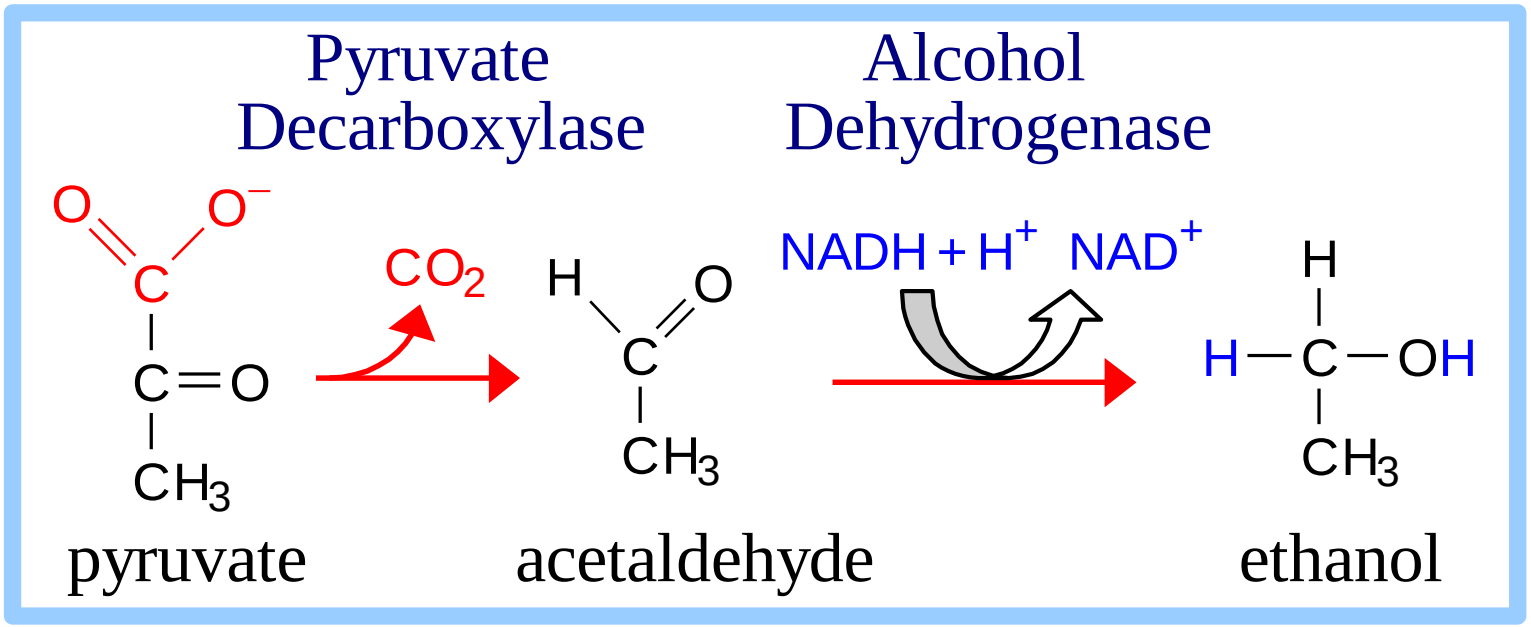
Lactate released to the **blood** may be taken up by other tissues, or by skeletal muscle after exercise, and converted via Lactate Dehydrogenase back to **pyruvate**, which may be oxidized in **Krebs Cycle** or (in liver) converted to back to **glucose** via gluconeogenesis



Lactate serves as a **fuel** source for **cardiac muscle** as well as **brain neurons**.

Astrocytes, which surround and protect neurons in the brain, **ferment glucose** to **lactate** and release it.

Lactate taken up by adjacent neurons is converted to pyruvate that is oxidized via Krebs Cycle.



Some anaerobic organisms metabolize pyruvate to **ethanol**, which is excreted as a waste product.

NADH is converted to **NAD$^+$** in the reaction catalyzed by Alcohol Dehydrogenase.

Glycolysis, omitting H^+:

glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

Fermentation, from glucose to lactate:

glucose + 2 ADP + 2 P_i → 2 lactate + 2 ATP

Anaerobic catabolism of glucose yields only 2 "high energy" bonds of ATP.

Glycolysis Enzyme/Reaction	$\Delta G^{o'}$ kJ/mol	ΔG kJ/mol
Hexokinase	-20.9	-27.2
Phosphoglucose Isomerase	+2.2	-1.4
Phosphofructokinase	-17.2	-25.9
Aldolase	+22.8	-5.9
Triosephosphate Isomerase	+7.9	negative
Glyceraldehyde-3-P Dehydrogenase & Phosphoglycerate Kinase	-16.7	-1.1
Phosphoglycerate Mutase	+4.7	-0.6
Enolase	-3.2	-2.4
Pyruvate Kinase	-23.0	-13.9

*Values in this table from D. Voet & J. G. Voet (2004) Biochemistry, 3rd Edition, John Wiley & Sons, New York, p. 613.

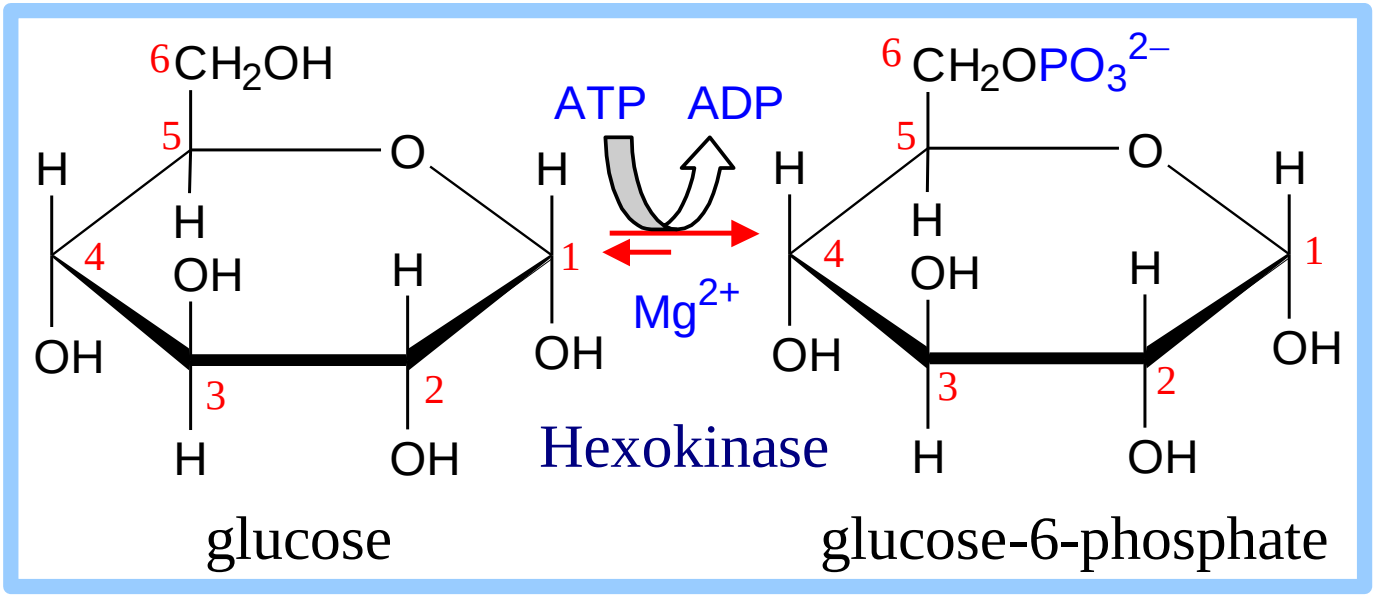
Flux through the Glycolysis pathway is **regulated** by control of 3 enzymes that catalyze **spontaneous** reactions:

Hexokinase, **Phosphofructokinase** & **Pyruvate Kinase**.

- **Local control** of metabolism involves regulatory effects of varied concentrations of pathway **substrates** or **intermediates**, to benefit the cell.
- **Global control** is for the benefit of the whole organism, & often involves **hormone-activated signal cascades**.

  Liver cells have major roles in metabolism, including maintaining blood levels various of nutrients such as glucose. Thus global control especially involves liver.

  Some aspects of global control by hormone-activated signal cascades will be discussed later.



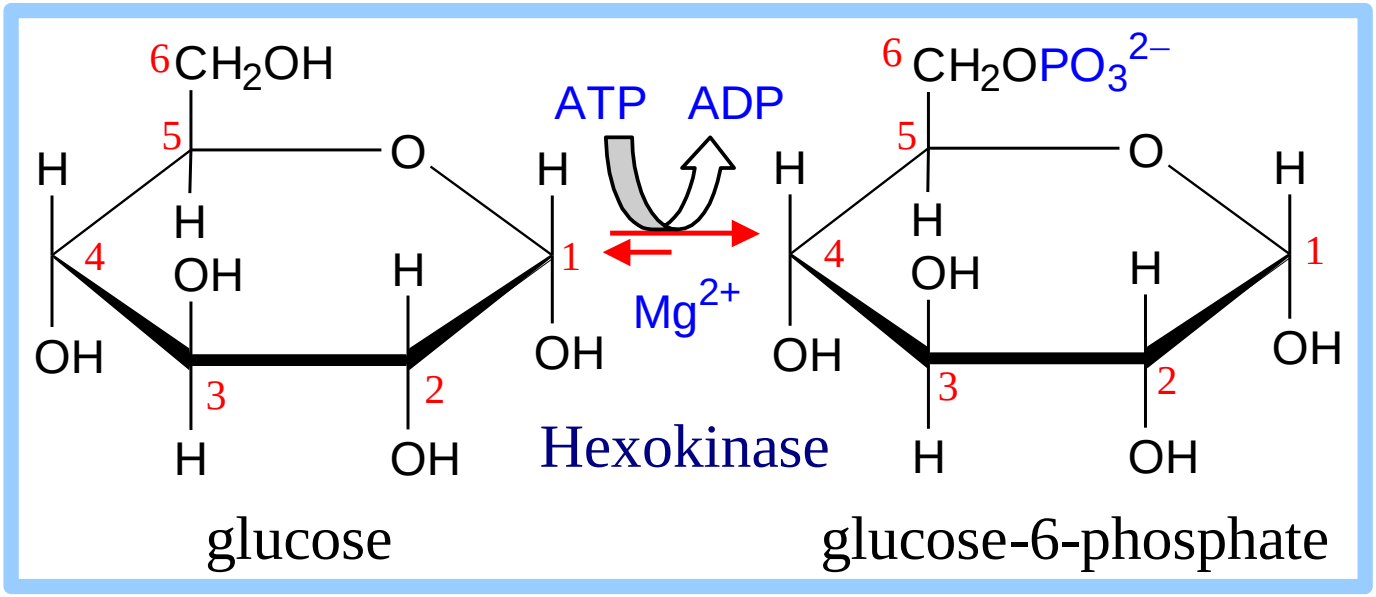
Hexokinase is **inhibited** by **product glucose-6-phosphate**:

- by **competition** at the **active site**
- by **allosteric** interaction at a **separate** enzyme site.

Cells **trap glucose** by **phosphorylating** it, preventing exit on glucose carriers.

Product inhibition of Hexokinase ensures that cells will not continue to accumulate glucose from the blood, if [glucose-6-phosphate] within the cell is ample.

Glucokinase is a variant of Hexokinase found in **liver**.

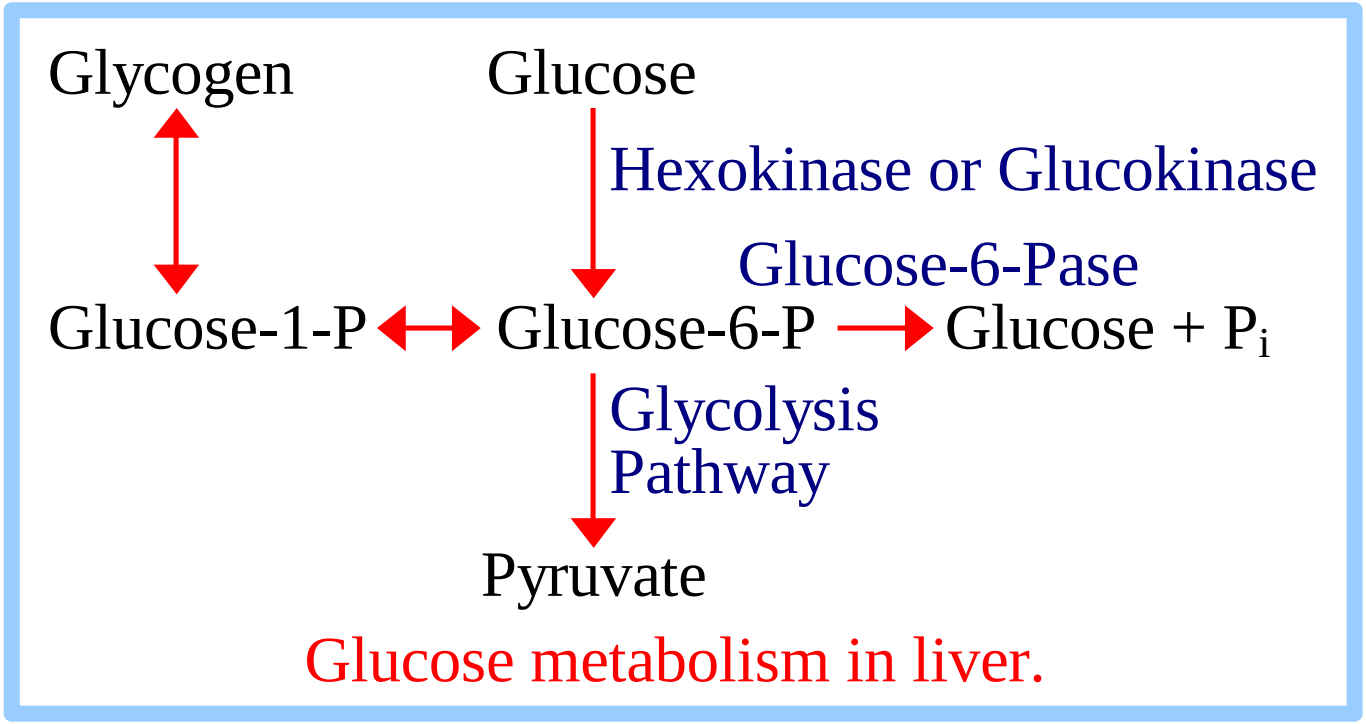


- **Glucokinase** has a **high K_M** for **glucose**.
  It is **active** only **at high [glucose]**.
- One effect of **insulin**, a hormone produced when blood glucose is high, is **activation** in liver of **transcription** of the gene that encodes the **Glucokinase** enzyme.
- Glucokinase is **not 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 **glucokinase regulatory protein** (**GKRP**).

  The ratio of Glucokinase to GKRP in liver changes in different metabolic states, providing a mechanism for modulating glucose phosphorylation.

Glucokinase, with high K_M for glucose, allows liver to store glucose as glycogen in the fed state when blood [glucose] is high.

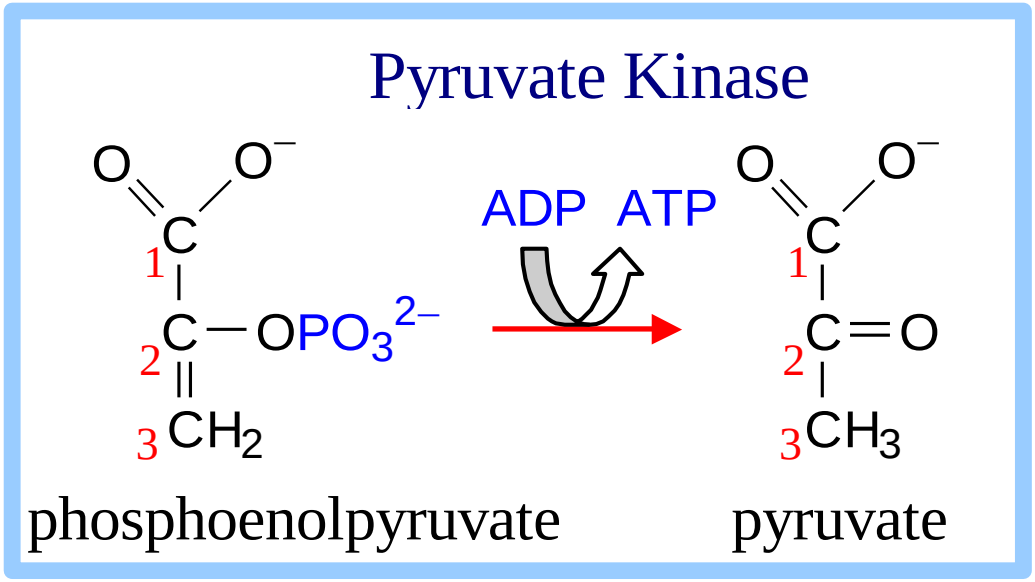


Glucose metabolism in liver.

Glucose-6-phosphatase catalyzes hydrolytic release of P_i from glucose-6-P. Thus **glucose** is **released** from the liver to the blood as needed to maintain blood [glucose].

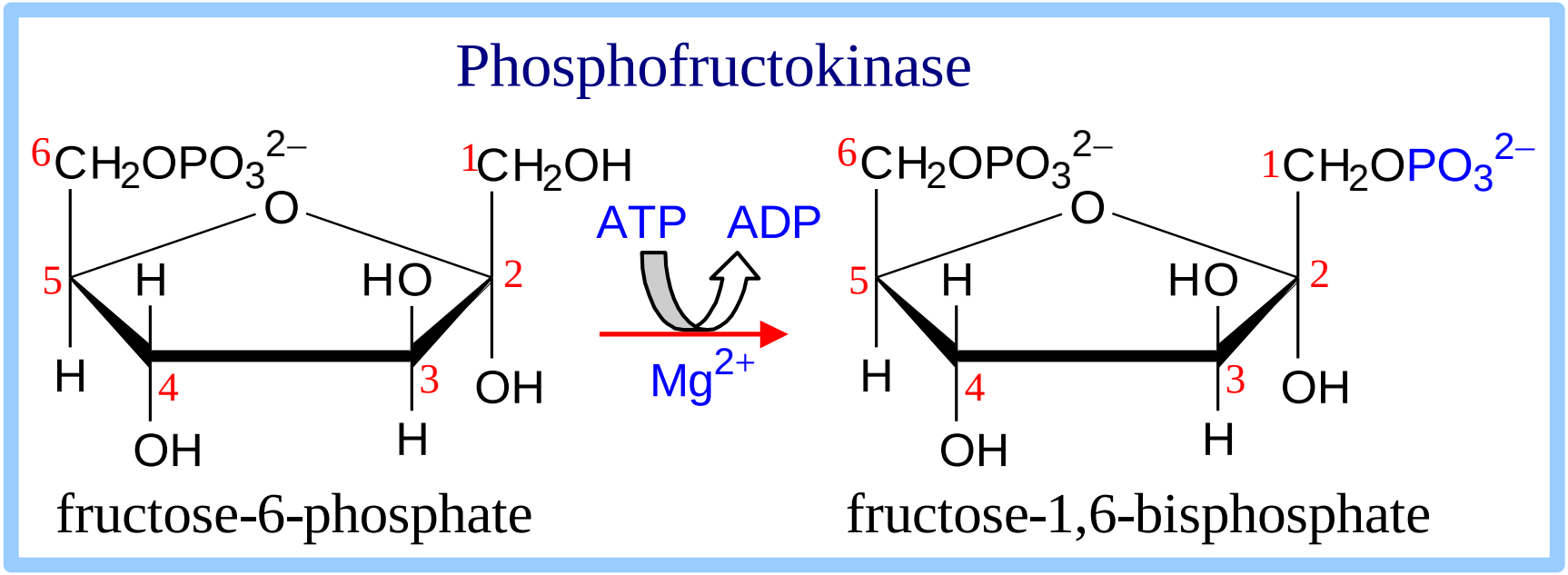
The enzymes Glucokinase & Glucose-6-phosphatase, both found in **liver** but not in most other body cells, allow the liver to control blood [glucose].

Pyruvate Kinase, the last step Glycolysis, is **controlled** in **liver** partly by modulation of the **amount** of **enzyme**.



High [glucose] within liver cells causes a transcription factor **carbohydrate responsive element binding protein** (**ChREBP**) to be transferred into the nucleus, where it activates **transcription** of the gene for Pyruvate Kinase.

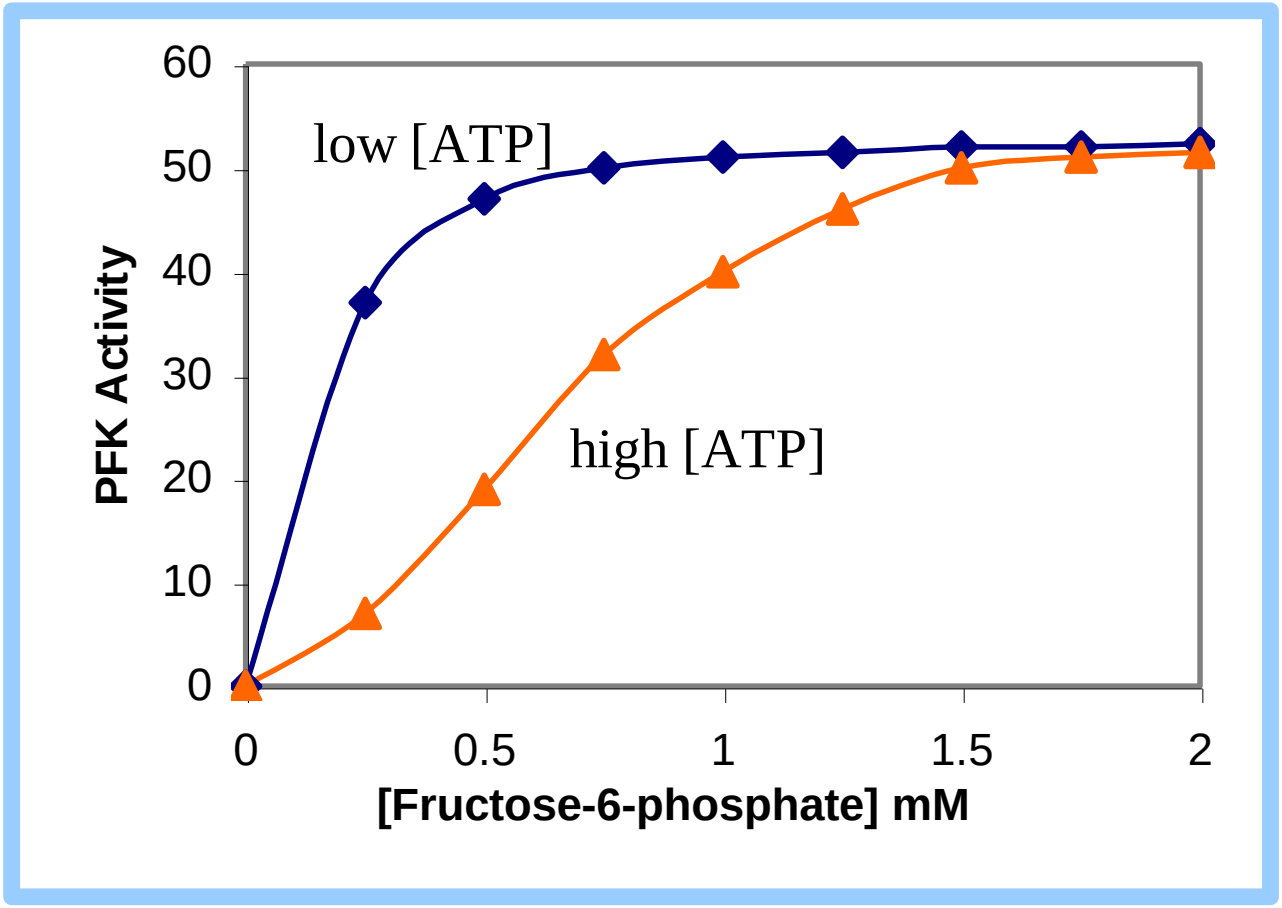
This facilitates converting **excess glucose** to **pyruvate**, which is metabolized to **acetyl-CoA**, the main precursor for synthesis of **fatty acids**, for long term energy storage.



Phosphofructokinase is usually the **rate-limiting step** of the Glycolysis pathway.

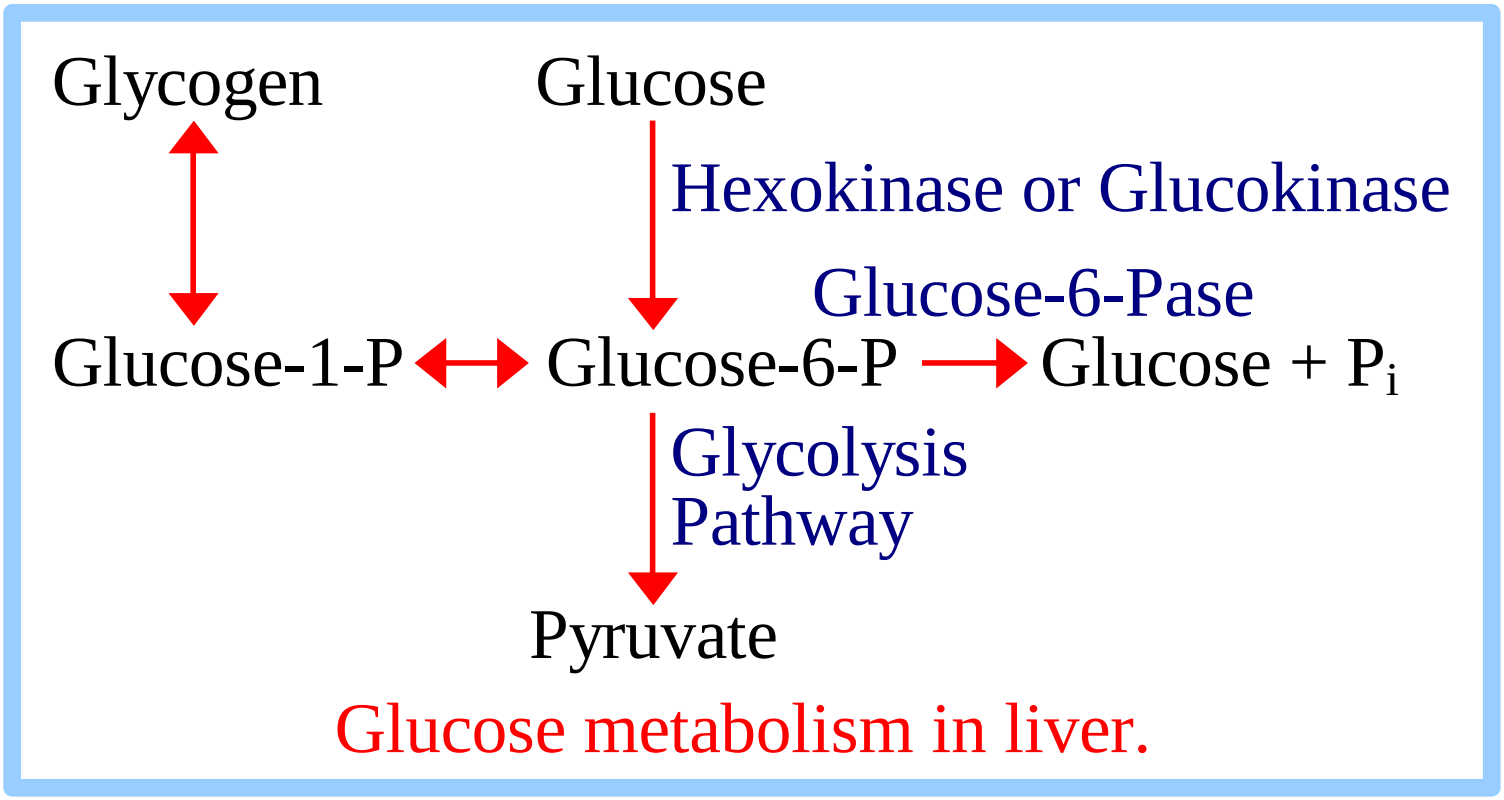
Phosphofructokinase is **allosterically inhibited by ATP**.

- 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. **Sigmoidal** dependence of reaction rate on [fructose-6-P] is seen.

AMP, present at significant levels only when there is extensive ATP hydrolysis, antagonizes effects of high ATP.



Inhibition of the Glycolysis enzyme Phosphofructokinase when [ATP] is high prevents breakdown of glucose in a pathway whose main role is to make ATP.

It is more useful to the cell to store glucose as glycogen when ATP is plentiful.

Glycolysis

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