

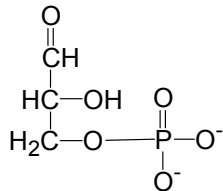
2nd Phase of Glycolysis

January 24, 2003

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In the preparatory phase of glycolysis two molecules of ATP have been invested. The hexose chain has been cleaved into two triose phosphates. The second phase contains the last five reactions of glycolysis and is called the payoff phase. It is called the payoff phase because in these five reactions two high energy phosphate bonds are produced. These 2 high energy phosphoryl groups are transferred to ADP to generate 2 molecules of ATP. It is important to remember that two molecules of glyceraldehydes 3-phosphate is generated per 1 molecule of glucose. The conversion of two molecules of glyceraldehydes 3-phosphate into two molecules of pyruvate is accompanied with the generation of 4 ATP molecules and 2 molecules of NADH. The net reaction for glycolysis is the production of 2 ATP.

Reaction 6 Oxidation and phosphorylation of glyceraldehydes 3-phosphate



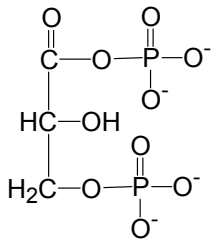
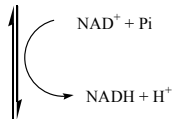
The first step of the second phase of glycolysis is the oxidation and phosphorylation of glyceraldehydes 3-phosphate to form 1, 3-bisphosphoglycerate.

The enzyme is **glyceraldehyde 3-phosphate dehydrogenase**.

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

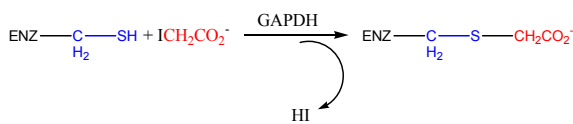
In erythrocytes $\Delta G = -1.29 \text{ kJ/mol}$

Another reaction near equilibrium.

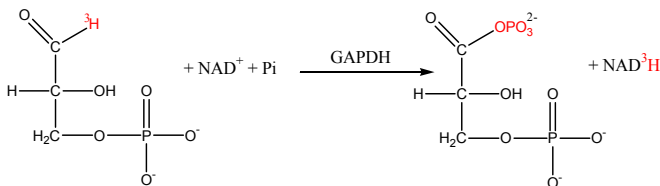


The oxidation of an aldehyde to the carboxylic acid is an exergonic process. This enzyme uses the free energy of aldehyde oxidation to synthesize a high energy acyl phosphate, 1, 3-bisphosphoglycerate.

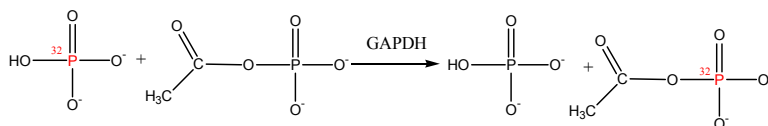
Fun facts about glyceraldehyde 3-phosphate dehydrogenase.



1. Iodoacetate, a reagent that alkylates cysteine residues, inactivates GAPDH. Iodoacetate reacts stoichiometrically with GAPDH. The confirmed presence of a carboxymethylcysteine residue demonstrates an active site cysteine residue that is essential for enzymatic activity.



2. GAPDH quantitatively transfer ³H from the C1 carbon of glyceraldehydes 3-phosphate to NAD⁺, establishing a direct hydride transfer.



3. GAPDH catalyzes ³²P exchange between inorganic phosphate and acetyl phosphate. Such isotope exchange is indicative of an acyl-enzyme intermediate.

Mechanism of GAPDH

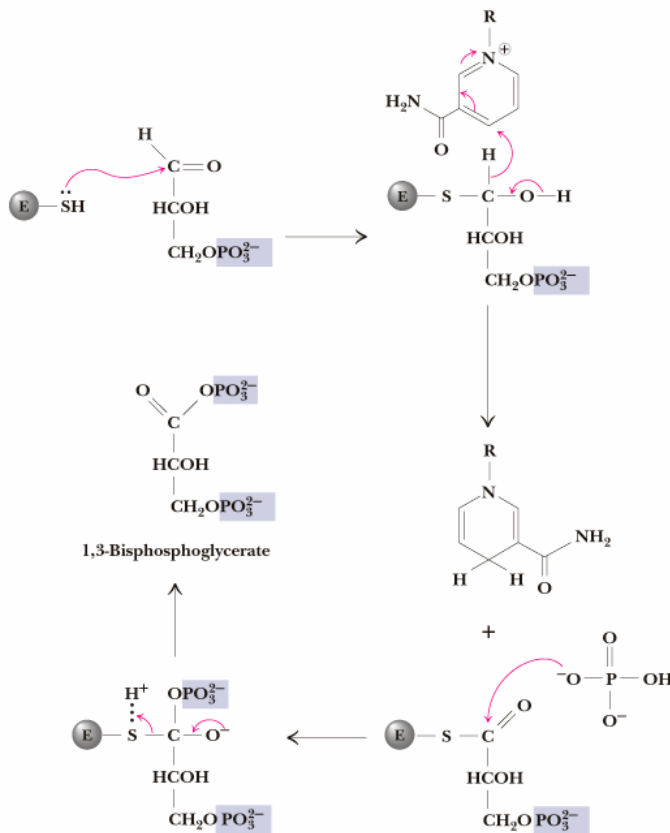
First the substrate G-3P binds to the enzyme.

The essential cysteine residue acts as a nucleophile and attacks the aldehyde carbonyl to form a thiohemiacetal intermediate.

Next the thiohemiacetal transfers a hydride ($2e^- + H^+$) to the active site bound NAD^+ to form NADH and a thioester intermediate.

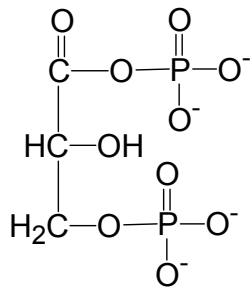
This NADH molecule dissociates from the active site and another NAD^+ molecule is bound.

The enzyme binds a molecule of inorganic phosphate which is the nucleophile that attacks the thioester. To regenerate the active enzyme's sulfhydryl and form 1, 3-



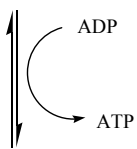
bisphosphoglycerate.

7th Reaction: Phosphoryl transfer from 1,3-bisphosphoglycerate.



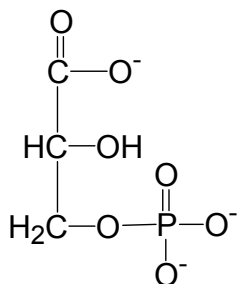
The enzyme phosphoglycerate kinase catalyzes the transfer of the high energy acyl phosphate to ADP. The result is the synthesis of two molecules of ATP. This enzyme pays off the ATP debt of the first phase of glycolysis.

$\Delta G^{o'} = -18.9 \text{ kJ/mol}$
 In erythrocytes $\Delta G = 0.1 \text{ kJ/mol}$

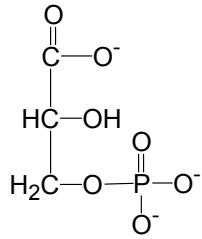


Another reaction near equilibrium.

Substrate level phosphorylation occurs when ADP is phosphorylated to form ATP at the expense of the substrate which is 1,3-bisphosphoglycerate in this case.



8th Reaction: Conversion of 3-phosphoglycerate into 2-phosphoglycerate.



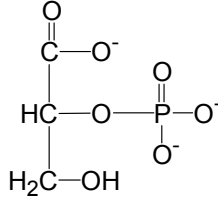
The enzyme **phosphoglycerate mutase** catalyzes the reversible shift of the phosphate ester between C2 and C3 of glycerate.

A **mutase** is an enzyme that catalyzes the migration of a functional group within the substrate molecule.

Mg^{2+} is required for enzymatic activity.

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

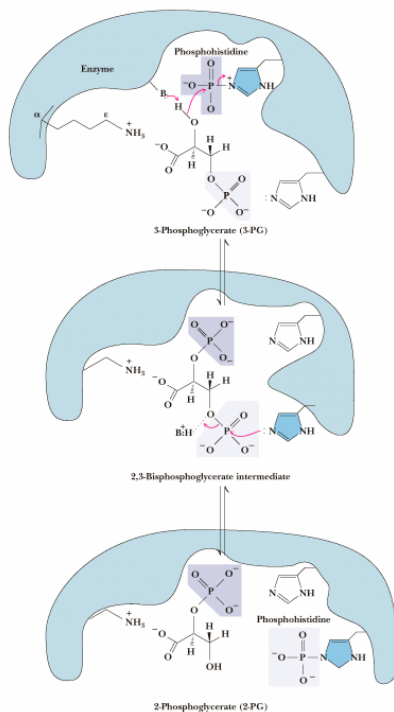
In erythrocytes $\Delta G = 0.83 \text{ kJ/mol}$



Another reaction near equilibrium.

This reaction is more complicated than it looks.

Phosphoglycerate mutase has a covalent phosphoenzyme intermediate and requires 2,3-bisphosphoglycerate as a cofactor. (Do you remember 2,3-bisphosphoglycerate, BPG, the allosteric effector of hemoglobin?)



The phosphoenzyme has a phosphoryl group covalently bound to an active site histidine residue.

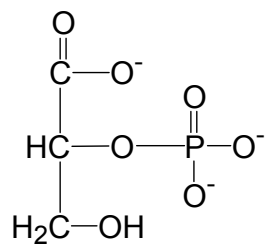
The phosphoenzyme binds 3-phosphoglycerate and transfer the phosphoryl group from the histidine to the C2 position of 3-phosphoglycerate to form 2,3-bisphosphoglycerate.

2,3-bisphosphoglycerate then transfers the C3 phosphoryl group to the active site histidine residue to regenerate the active phosphoenzyme and produce 2-phosphoglycerate.

Once in every 100 turnovers, the intermediate 2,3-bisphosphoglycerate escapes from the enzyme, leaving an inactive dephosphorylated enzyme.

The unphosphorylated enzyme binds 2,3-bisphosphoglycerate and transfers the C3 phosphoryl group to the histidine reactivating the enzyme and producing 2-phosphoglycerate. For this reason, the enzyme requires a small amount of 2,3-BPG as a cofactor for maximal activity.

Reaction 9: Dehydration of 2-phosphoglycerate



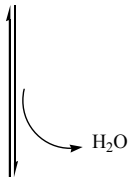
Enolase is the enzyme that catalyzes the reversible elimination reaction of water to convert 2-phosphoglycerate into phosphoenolpyruvate.

Mg^{2+} required for enzymatic activity.

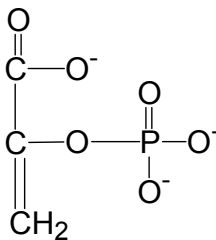
$\Delta G^{\circ\prime} = 7.5 \text{ kJ/mol}$

In erythrocytes $\Delta G = 1.1 \text{ kJ/mol}$

Another reaction near equilibrium

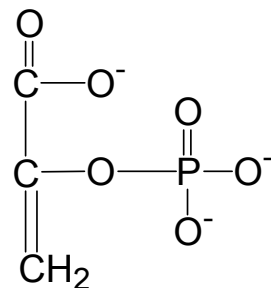


Enolase dehydrates the substrate to form a high energy phosphoenol. The standard change in free energy for the hydrolysis of phosphoenolpyruvate is a -62 kJ/mol .



This enzyme is strongly inhibited by fluoride in the presence of phosphate. Fluoride reacts with phosphate to form fluorophosphates (FPO_3^{2-} which complexes with the Mg^{2+} ion located in the active site of the enzyme.

Reaction 10: Transfer of the phosphoryl group of phosphoenolpyruvate.



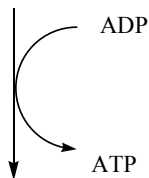
The last step of glycolysis is the transfer of the phosphoryl group of phosphoenol pyruvate to ADP to form ATP and pyruvate. The enzyme that catalyzes this reaction is pyruvate kinase.

This enzyme binds MgADP or MgATP^{2-}
I.e Mg^{2+} required for enzymatic activity.

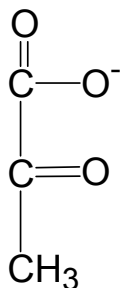
$\Delta G^{\circ\prime} = -31.4 \text{ kJ/mol}$

In erythrocytes $\Delta G = -23.0 \text{ kJ/mol}$

Far from equilibrium.



Since 2 PEP are formed for every glucose molecule that enters the glycolytic pathway, 2 ATP molecules are formed in this step. The ATP debt generated during phase 1 of glycolysis was paid by the formation of ATP by the substrate level phosphorylation of ADP by 1,3-bisphosphoglycerate. The 2 ATP molecules produced here the payoff for glycolysis.



The large negative ΔG of this reaction makes this enzyme a target for regulation. Pyruvate kinase has binding sites for a number of allosteric effectors.

\uparrow AMP

\uparrow Fructose 1,6-bisphosphate

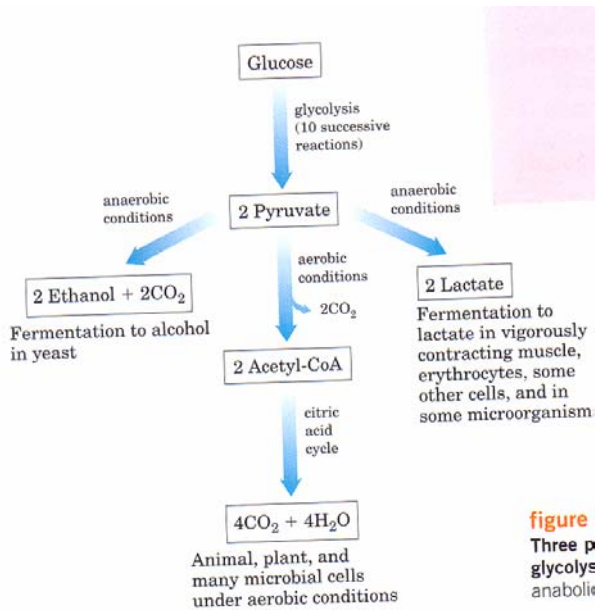
\downarrow ATP

\downarrow Acetyl-CoA

\downarrow Alanine

In addition to the allosteric effectors, pyruvate kinase is regulated by covalent modification. Hormones such as glucagon activate a cAMP-dependent protein kinase which transfers the γ -phosphate of ATP to the pyruvate kinase. The phosphorylated pyruvate kinase is more strongly inhibited by ATP and alanine. The K_m for PEP for the phosphorylated enzyme is also increased to the point that at the steady state physiological concentrations of PEP, the enzyme is completely inactive.

Fates of Pyruvate



The two molecules of pyruvate and 2 molecules of NADH produced from one molecule of glucose during glycolysis have three possible metabolic fates.

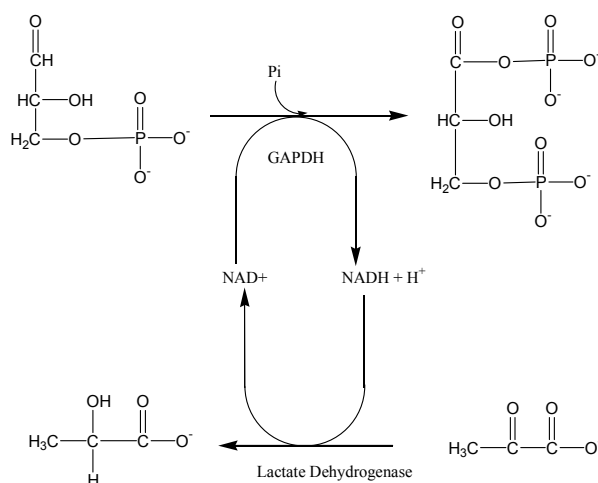
Under aerobic conditions, pyruvate is oxidized, with the loss of CO_2 to produce acetyl-CoA and NADH. Acetyl-CoA then enters the citric acid cycle where it is completely oxidized into CO_2 and H_2O . Under aerobic conditions the NADH produced from glycolysis and the citric acid cycle are reoxidized into NAD^+ in the mitochondrial electron transport chain.

Under anaerobic conditions NADH accumulates as a product of GAPDH catalyzed sixth step of glycolysis. NAD^+ is required as a substrate for GAPDH and soon becomes limiting.

figure Three p glycolyse anaboli

In order to maintain production of ATP via the glycolytic pathway under anaerobic conditions, NAD^+ has to be regenerated from NADH. There are two possible anaerobic pathways.

1.) Homolactic acid fermentation.



Under anaerobic conditions, such as when muscle cells are vigorously working, pyruvate is reduced to lactic acid to regenerate NAD^+ . This process is called **homolactic fermentation**.

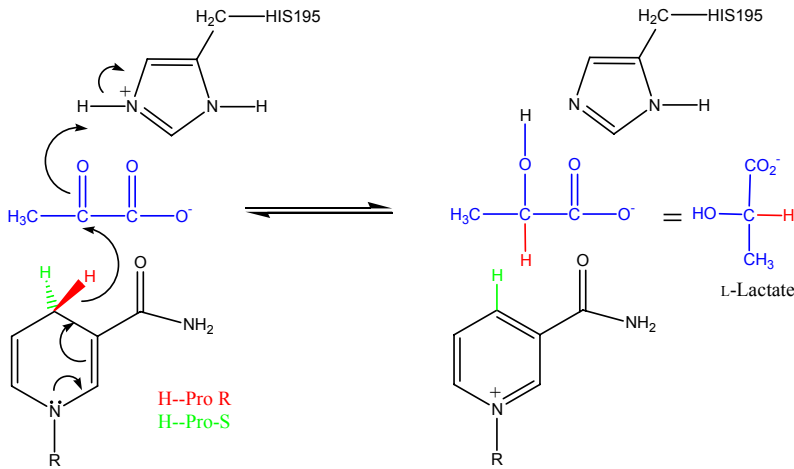
For lactate dehydrogenase

$$\Delta G^{\circ\prime} = -25.1 \text{ kJ/mol}$$

In erythrocytes $\Delta G = -14.8 \text{ kJ/mol}$

Speaking of erythrocytes. Red blood cells do not have mitochondria. They convert glucose into 2 molecules of lactate even under aerobic conditions.

Other tissues (retina, brain) convert glucose into two molecules of lactate under aerobic conditions.



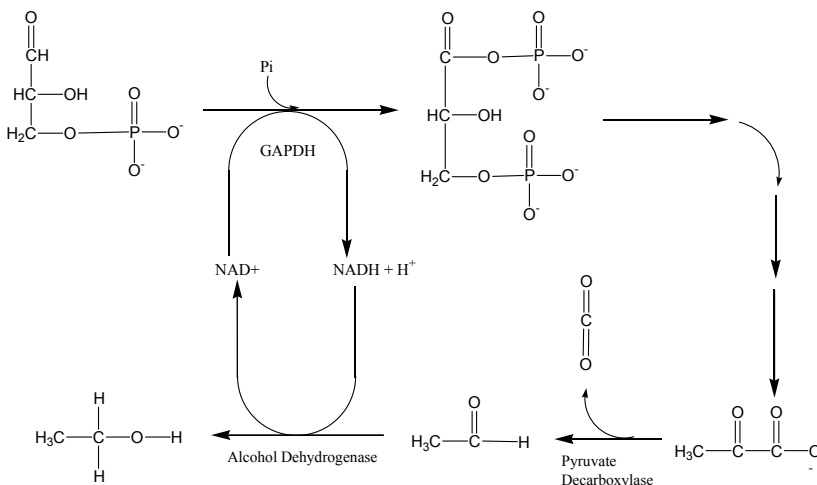
Lactate dehydrogenase, LDH catalyzes the reduction of pyruvate to lactate with absolute stereospecificity.

The Pro-R hydrogen of NADH is transferred to the pyruvate to form L-lactate.

The mechanism of LDH is shown to the left.

The pro-R hydride is transferred from NADH to C2 of pyruvate with the concomitant transfer of a proton from the imidazolium His-195.

2.) Alcoholic fermentation



Under anaerobic conditions some plant tissues, invertebrates, protists, and microorganisms (yeast) regenerate NAD⁺ by alcoholic fermentation. Pyruvate and NADH are converted into ethanol, CO₂ and NAD⁺.

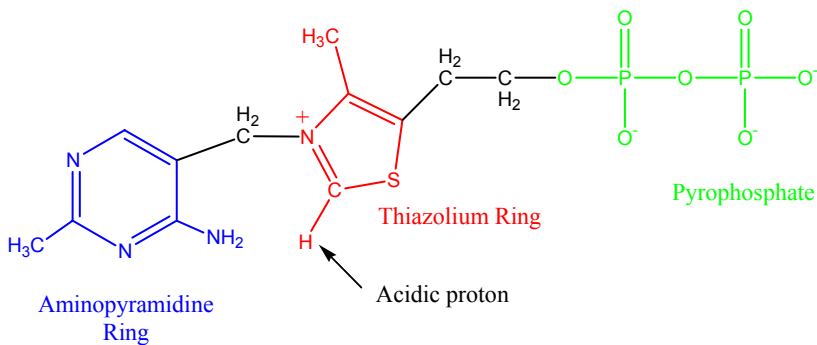
Yeast under anaerobic conditions regenerates NAD⁺ by alcoholic fermentation. This is an important reaction in making beer, wine and spirits. In addition the CO₂ produced by alcoholic fermentation leavens bread.

The conversion of pyruvate into ethanol is a two step process. The first step involves the decarboxylation of pyruvate by the enzyme **pyruvate decarboxylase**.

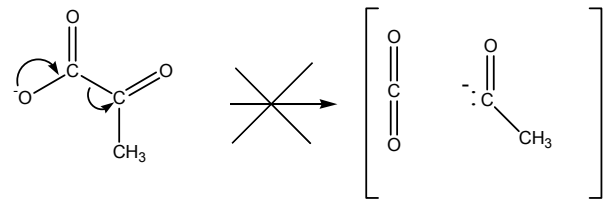
This enzyme requires the coenzyme **thiamine pyrophosphate, TPP**.

This cofactor is bound tightly by the enzyme.

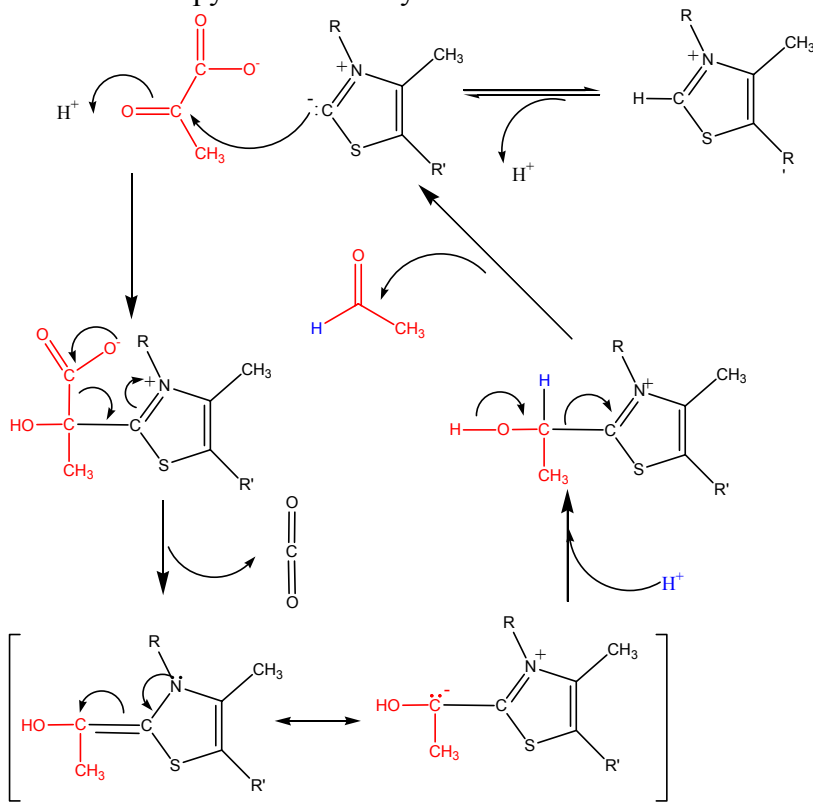
Thiamine pyrophosphate



Thiamine pyrophosphate functions as an electron sink to stabilize the build up of negative charge of the carbonyl carbon atom in the transition state.

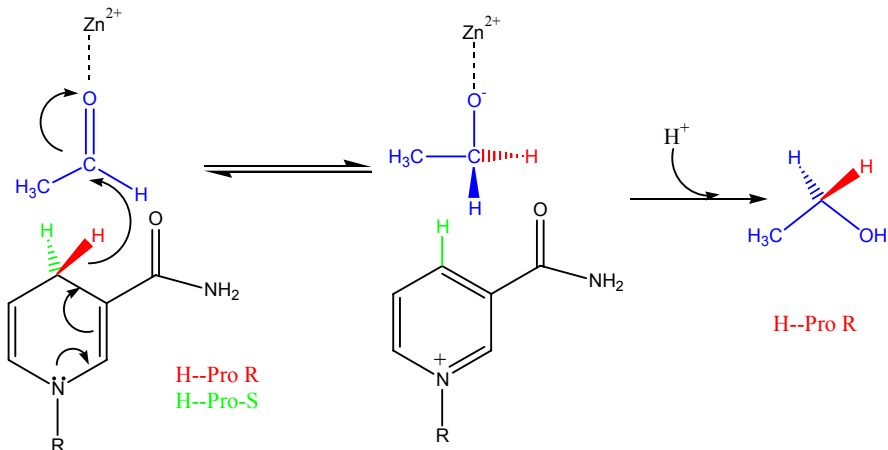


Mechanism of pyruvate carboxylase



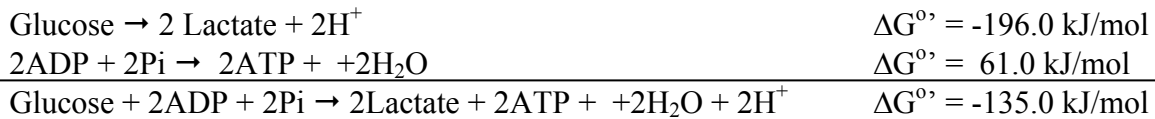
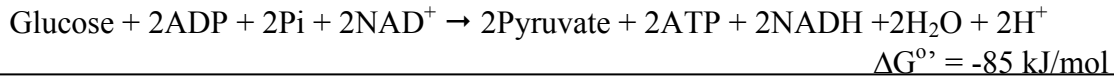
The next step of alcoholic fermentation is the reduction of acetaldehyde by NADH to produce ethanol and NAD^+ . The enzyme that does this reduction is **alcohol dehydrogenase, ADH**. Yeast alcohol dehydrogenase contains an active site Zn^{2+} ion. This zinc polarizes the carbonyl bond of acetaldehyde and to stabilize the negative charge that is built up in the transition state. ADH is stereospecific it transfers the pro-R hydride of NADH to the pro-R position of ethanol.

Yeast alcohol dehydrogenase



In the Mammalian liver there is ADH that catalyzes the opposite reaction. The oxidation of ethanol in to acetaldehyde. This enzyme has two Zn^{2+} ions in the active site although only one of the zincs participates directly in catalysis through the same general mechanism.

Energetics of Homolactic acid Fermentation



Efficiency of Homolactic acid Fermentation.

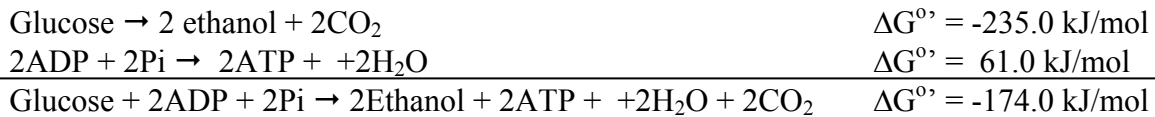
2 ATP's produced 61 kJ/mol

Standard free energy change in converting glucose into two molecules of lactate $\Delta G^{\circ\prime} = -196.0 \text{ kJ/mol}$.

Efficiency = $100 \times 61/196 = 31\%$

Under the steady state concentrations of the cell the efficiency is greater than 50%.

Energetics of alcoholic fermentation.



Efficiency = $100 \times 61/235 = 26\%$

Under the steady state concentrations of the cell the efficiency is greater than 50%.

Glycolysis is used for rapid ATP production.

The rate of ATP formation in anaerobic glycolysis is 100 times faster than ATP production by oxidative phosphorylation in the mitochondria. When tissues are rapidly consuming ATP, they generate it almost entirely by anaerobic glycolysis.

Two type of muscle fibers **Fast twitch** and **Slow twitch**.

Fast-twitch fibers are capable of short burst of rapid activity. The cells that compose these fibers are almost completely devoid of mitochondria. They generate nearly all of their energy by anaerobic glycolysis. They are easily identifiable as white fibers.

Slow-twitch fibers contract slowly and steadily. They are rich in mitochondria and obtain most of their energy by oxidative phosphorylation. The high concentration of mitochondria give these muscle fibers a red color to the heme containing cytochromes.

The flight muscles of birds are an illustrative example. The flight muscles of migratory birds such as ducks and geese are rich in slow twitch fibers and therefore these birds have dark breast meat. In contrast the flight muscles of the land loving birds such as chickens and turkeys are used only for short burst of flight to escape danger. Their flight muscles are composed mainly of fast twitch fibers that give them white breast meat.

Sprinters muscles mainly fast-twitch fibers.

Marathon runners mainly slow-twitch fibers.