Biochemistry - I

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Lecture #26
Metabolism - II

We continue our glycolyis, we started of yesterday where we considered different metabolic processes that actually go on in the body. And if we lo at the first slide here, the overall catabolic processes lo at the break down of lipids polysaccharides and proteins. Now the break down of lipids gets into components of fatty acids and glycerol. The polysaccharides break down to monosaccharide and the proteins breakdown to amino acids.

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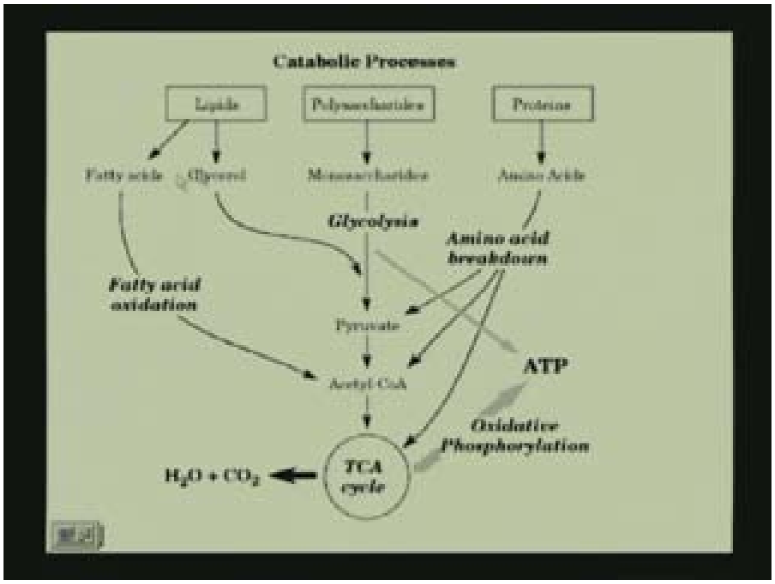

Catabolic Processes

Lipids
Polysaccharides
Proteins
Fatty acids
Glycerol
Monosaccharides
Amino Acids
Glycolysis
Amino acid breakdown
Fatty acid oxidation
Pyruvate
Acetyl-CoA
ATP
Oxidative Phosphorylation
$H_2O + CO_2$
TCA cycle

Now in the anabolic processes we have these broken down amino acids and other factors that actually get on into building up the other macro molecules that are required for our bodily functions. Now what we are interested in is the break down of the monosaccharide particularly the processes of glycolysis that takes glucose and breaks it down into pyruvate and later on we will see how this pyruvate then gets on into the tricarboxylic acid cycle or the kreb cycle to finally get to water and carbon dioxide and an off shoot of that is the production of ATP where we studied oxidative phophorylation in the different complex processes that require a number of electron transfer cofactors as well as certain enzymes.

The overall equation for glycolysis is the break down of glucose into 2 pyruvate. Now what we have here is we see how ATP is produced. As we continue with all the steps we will see how ATP is produced in some of the steps but in the first set that we did, we found ATP consumption.

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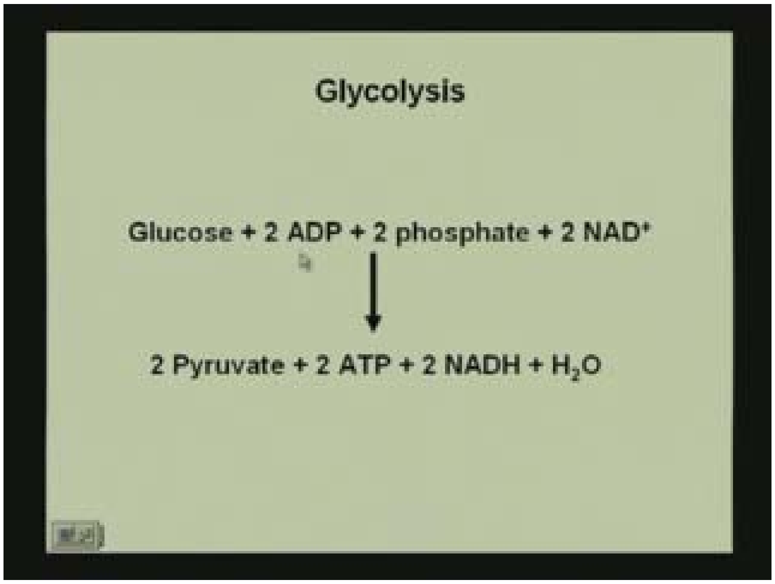

Glycolysis

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

This is [Refer Slide Time: 02:34] the number of steps that we considered in our last class. We had glucose going to glucose-6-phosphate, the enzyme being hexinase. We have to remember that a kinase is a transferase that transfers a phosphate group, so in this process we had the break down of ATP to ADP and the phosphate was transferred to glucose. This then went on to form the ketose from aldose so we had fructose-6- phosphate that had the enzyme phosphoglucose isomerase acting on it because this is an isomer the aldose and the ketose that we have here. The fructose-6- phosphate then when on to form fructose1, 6-bisphosphate where we required another ATP to be broken down and the enzyme used there was another kinase but in this time it was phosphofructinase.

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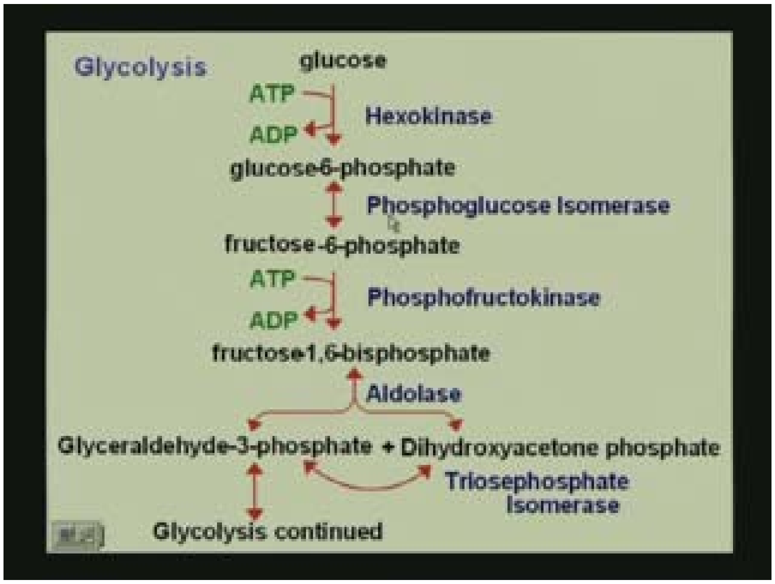

Glycolysis

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

After this particular step we have aldolase come into the picture, aldolase is actually what breaks up the 6 carbon membered ring into 2, 3 carbon membered rings where we have glyceraldehyde-3-phosphates and dihydroxyacetone phosphate. Now these two are intertransferable by the enzyme triose phosphate isomerase and we also learnt that the equilibrium of this enzyme is such that the dihydroxy acetone phosphate is preferred to be formed in larger quantities but to shift the equilibrium to the right where glyceraldehyde -3- phosphate will be utilized because that is what continues the glycolysis process. So we have to have glyceraldehyde-3- phosphate that is going to the glycolysis or continue with the breakdown and we have to remember here that we now have 2, 3 carbon units instead of the 6 carbon unit that we started off with.

So this [Refer Slide Time: 04:32] is our step number 6, if you remember we reached glyceraldehyde-3- phosphate after the action of aldolase on fructose 1, 6 bisphosphate. So our next step is the formation of 1, 3 bisphosphoglycerate from glyceraldehyde-3-phosphate. So we are now adding a phosphate but we have not used ATP in this step, we are using P_i. So the reaction here is glyceraldehyde-3- phosphate plus NAD plus P_i in a reversible step giving you 1, 3 bisphosphoglycerate plus NADH plus H plus.

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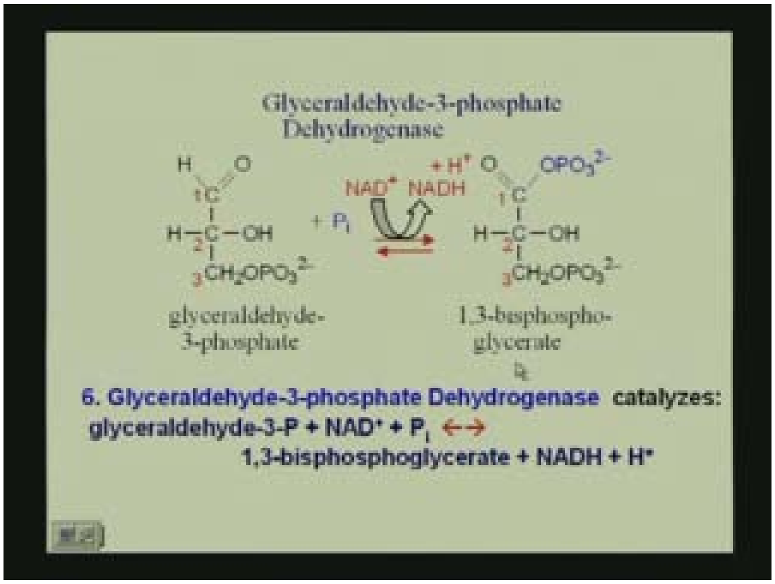

Glyceraldehyde-3-phosphate Dehydrogenase

6. Glyceraldehyde-3-phosphate Dehydrogenase catalyzes:
glyceraldehyde-3-P + NAD$^+$ + P$_i$ ↔
1,3-bisphosphoglycerate + NADH + H$^+$

So this is our sixth step where what has been done is we have added a phosphate using a NAD plus forming NADH, in a reversible step. Now in this case what happens this is the only step in glycolysis where NAD plus is actually reduced to NADH. In this step that uses glyceraldehyde-3- phosphate dehydrogenase because you have this hydrogen being removed from the aldehyde group and you have a phosphate introduced by the P_i that comes in as a factor to form 1, 3 bisphosphoglycerate. And what we have here now is if you notice we are going to actually create an acid from this because this now as a form where we have C double bond OPO_3 2 minus. This was initially an aldehyde and we are gradually getting to an acid.

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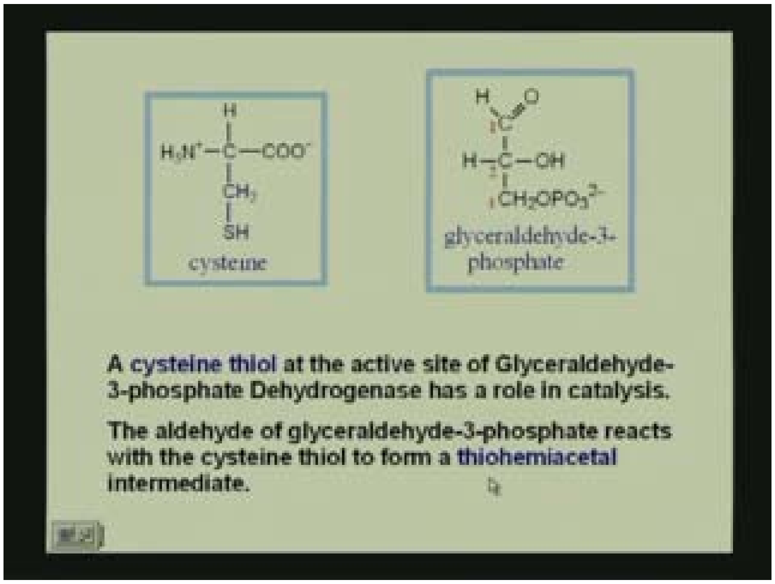

A cysteine thiol at the active site of Glyceraldehyde-3-phosphate Dehydrogenase has a role in catalysis.

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

Now what is going to happen is in this case there is a cysteine thiol, all of us know that in the cysteine residues we have the SH thiol group. Now what happens is the cysteine thiol is present at the active site of the glyceraldehyde-3- phosphate dehydrogenase and that actually forms an intermediate with the acetaldehyde of the glyceraldehyde-3- phosphate, the aldehyde that reacts with the cysteine thiol to form what is called thioemiacetal. So what happens here like we learnt yesterday in one of the reactions we had lysine come into the picture, here we have cysteine we will also seen one of the enzymes has hystedene. So these are the particular side chain of the enzymes that are going to take part in the overall reactions for the transformation to occur.

Now in this [Refer Slide Time: 07:30] case what we have, here is our enzyme with the cysteine group attached to it. Anyway what we have here, you can see the enzyme has the SH group attached to this. Now what is happening here, this SH creates a thioemiacetal intermediate by reacting with the aldehyde of the glyceraldehyde-3- phosphate. So this is our glyceraldehyde. So this is carbon number 1, carbon number 2, carbon number 3 because now remember we have broken down our glucose into a 3 carbon system.

So the enzyme, the cysteine group that is present in the active site of glyceraldehyde-3- phosphate dehydrogenase is forming a thioemiacetal intermediate with the aldehyde of the glyceraldehyde-3- phosphate. So now we have this linked to the enzyme, now this part is linked to the enzyme then NAD plus is reduced to NADH in the process we get an acyl thioester intermediate where is this thioester? You see this CH OH is now C double bond O and the S is part of the cysteine. So it becomes a thioester an acyl thioester intermediate.

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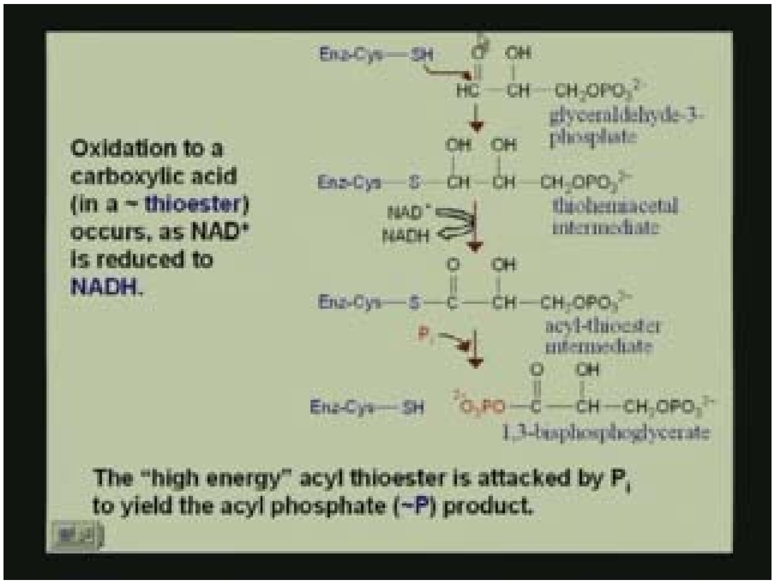

Oxidation to a carboxylic acid (in a ~ thioester) occurs, as NAD⁺ is reduced to NADH.

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

Now what happens is then the P_i that is the phosphate comes and cleaves the S C bond and your enzyme is regenerated. And in the event what happens, the phosphate adds on to the first carbon atom. So now you have 1, 3 bisphosphoglycerate. Is that clear? So what happens is we have this acyl thioester that is attacked by the P_i which actually cleaves the substrate or rather in this case it is now become the product and regenerates the enzyme. So again we have enzyme cysteine with SH that now is ready to act upon another glyceraldehyde-3- phosphate.

So basically what we have here is from glyceraldehyde-3- phosphate with the help of this cysteine of the enzyme we have the formation of thioemiacetal intermediate that then forms an acyl thioester intermediate which then is attacked by P_i which yields 1, 3 bisphosphoglycerate and regenerates the enzyme. So that [Refer Slide Time: 10:44] is our step. Now we have landed up with 1, 3 bisphosphoglycerate, now what happens is this phosphate is taken up by ADP to produce ATP. So we have now the first reaction where we have produced ATP in our glycolysis step. So ADP, one of the phosphates from 1, 3 bisphosphoglycerate is the first one is lost to ADP which produces ATP, in the event it forms 3 phosphoglycerate.

So we now have an acid here not an aldehyde at carbon number 1 and all of this remember is enzymatic. So initially we had, what was the first thing that we had here? We had glyceraldehyde-3- phosphate. So this was our aldehyde, I have to create pyretic acid so I have to create an acidic group at position number 1. The first step in doing that is the formation of 1, 3 bisphosphoglycerate. In the formation of 1, 3 bisphosphoglycerate the enzyme glyceraldehyde-3- phosphate dehydrogenase utilizes a cysteine thiol to bring about the reaction. And in the next step we lose the phosphate to ADP which produces ATP and we have 1, 3 bisphosphoglycerate plus ADP go to 3 phosphoglycerate plus ATP.

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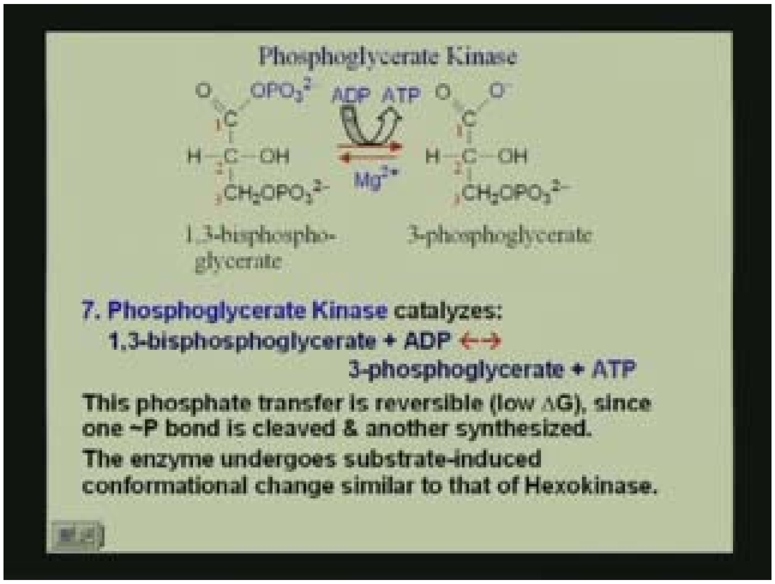

7. Phosphoglycerate Kinase catalyzes:

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.

The next step is a mutase, if you remember we considered in our last class all the enzymes that are actually going to take part in the glycolysis steps. The mutase shifts the phosphate from one position to the other. So the mutase enzyme shifts a myt from one carbon atom to another, in this case the 3 phosphor is shifted to the second carbon, so the product is 2- phosphoglycerate.

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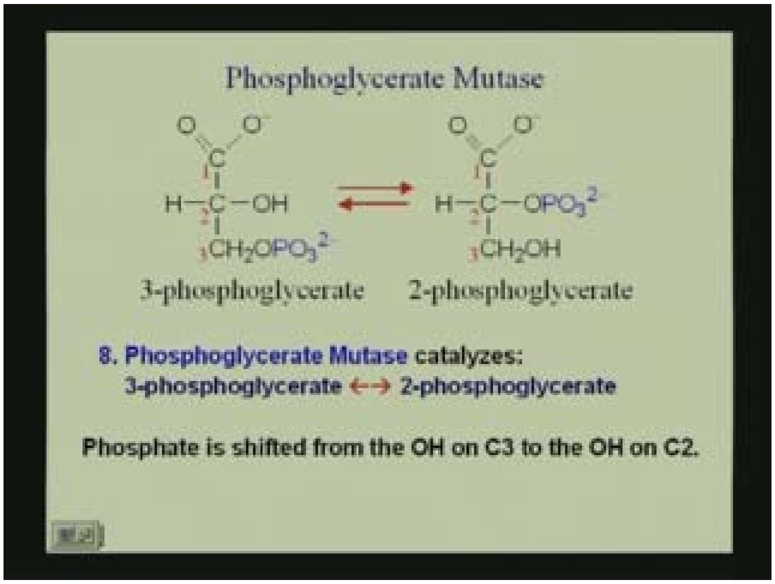

Phosphoglycerate Mutase

8. Phosphoglycerate Mutase catalyzes:
3-phosphoglycerate ↔ 2-phosphoglycerate

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

So instead of 3 phosphoglycerate after the action of this enzyme phosphoglycerate mutase which shifts the phosphate myt from the third carbon atom to the second carbon atom.

So the phosphate is shifted from the OH on C_3 to the OH on C_2, so we have a shift here [Refer Slide Time: 13:44]. Now in this case from 3- phosphoglycerate to 2- phosphoglycerate the enzyme which is phosphoglycerate mutase has a histidine. And this histidine is present again in the active side of the enzyme and what it does is, it helps in the phosphate transfer by accepting the phosphate and then donating it again. So the mechanism is such that the histidine accepts the phosphate from 3-phosphoglycerate and then donates it back but to the second carbon atom so this would obviously depend upon the position of where the carbon atoms are actually located in the active site of phosphoglycerate mutase.

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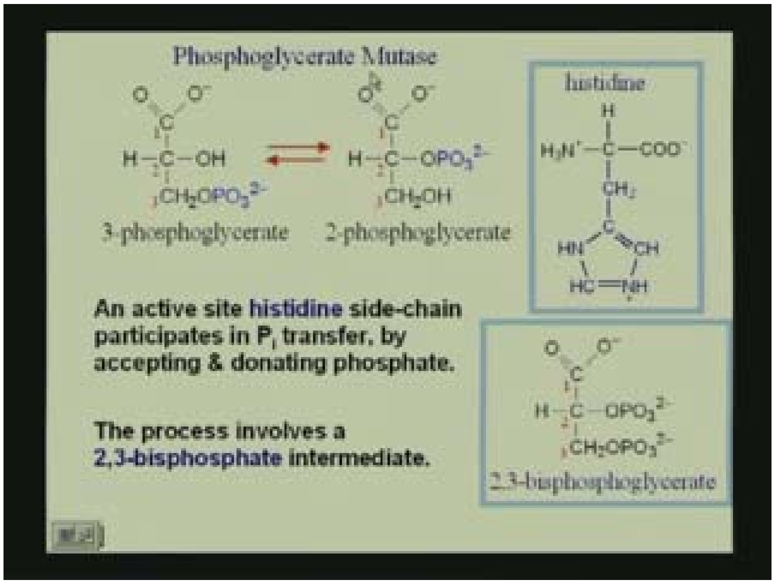

Phosphoglycerate Mutase

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

The process involves a 2,3-bisphosphate intermediate.

And in the intermediate that we have here, we have an additional actually 2, 3 bisphosphoglycerate and this finally looses the phosphate that is the third carbon atom into becoming 2-phosphoglycerate. So now we have already formed the acid we are gradually getting to the step where we are going to form an enol and then a pyruvate.

Now in the ninth step we have enolase, enolase actually we have now reached 2-phosphoglycerate, we had 3-phosphoglycerate we have now formed 2-phosphoglycerate by an enzyme called mutase. After 2- phosphoglycerate we have now an enolate intermediate. What is that enolate intermediate? It has formed with the loss of H plus here which has formed double bond at this position between the carbon atom 1 and 2 we have a double bond formation. Then with the loss of OH we have a double bond between carbons 2 and 3.

So this negative charge of the oxygen will come back here, this forms a double bond here and the OH is lost. Is the mechanism clear? So we have the O come back here to form the double bond then this double bond shifts here, the OH is lost. So we get phosphoenolpyruvate, so we are gradually getting to our final step which is going to be the formation of pyruvate from glucose.

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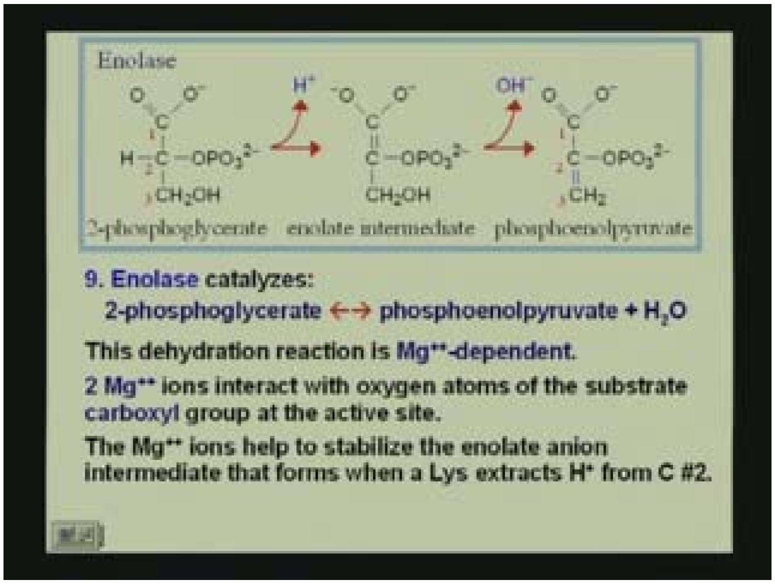

9. Enolase catalyzes:
2-phosphoglycerate ←→ phosphoenolpyruvate + H_2O

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.

So we have in our ninth step enolase that catalyses the formation of phosphoenolpyruvate from 2-phosphoglycerate which is actually nothing but the dehydration reaction. So essentially you are losing this hydrogen and this OH into forming a double bond between carbon atoms 2 and 3, that is essentially what is happening. So you have CH_2 double bond C with a phosphate attached and C O minus a carboxylate group. So essentially you are having a dehydration where you are loosing this hydrogen and this OH and you are forming a double bond. Now what else do you have to loose to form the pyruvate? The phosphate, so that will be our next step but there is some other notes here where we have the dehydration reaction is magnesium depended.

The 2 magnesium ions interact with oxygen atoms of the substrate carboxyl group at the active site. So what happens is we have a magnesium interacting with the oxygen atoms of the carboxylic group of 2- phosphoglycerate that helps stabilize. We have two O minus so what happens is the magnesium ions stabilize this enolate ion and then we have an extraction of the hydrogen from carbon number 2 when a Lysine actually helps in doing that. So the active site in this case would be the presence of Lysine and the presence of 2 magnesium ions that would help stabilize the enolate intermediate.

So our next step now is the formation of pyruvate where we have to loose the phosphate. This is the last step where we have formed pyruvate, pyruvate is CH_3 C double bond O C OH that is pyruvic acid. So we have phosphoenolpyruvate that was formed from 2 phosphoglycerate, from 2- phosphoglycerate we formed phosphoenolpyruvate and we now know that to form pyruvic acid we have to have CH_3 C double bond O COOH or other pyruvate would be CH_3 C double bond O COO minus. So we have to loose this phosphate. And who takes up the phosphate? ADP.

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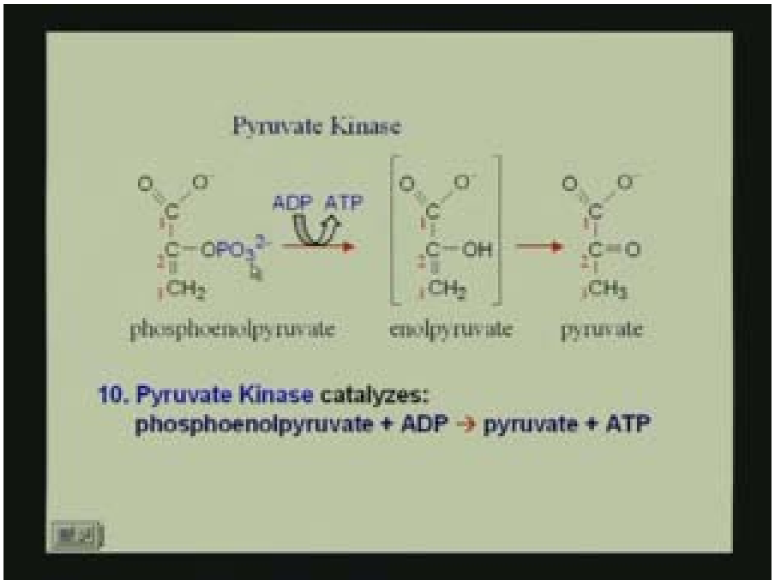

Pyruvate Kinase

10. Pyruvate Kinase catalyzes:
phosphoenolpyruvate + ADP → pyruvate + ATP

So now we form another ATP here in this step of glycolysis. And the enzyme here is pyruvate kinase that is shifting the phosphate, remember the kinase shifts the phosphate. It is taking the phosphate from phosphoenolpyruvate and giving it to ADP and forming ATP. The intermediate is enol pyruvate that actually then forms the pyruvate. So our last step is phosphoenolpyruvate plus ADP going to pyruvate plus ATP. So eventually we broken down the glucose and we have finally formed pyruvate. This pyruvate is then later on going into through acetylCoA it is going to go into the tricarboxylic acid cycle and from there we will have the production of carbondioxide and water which you will see in a later class.

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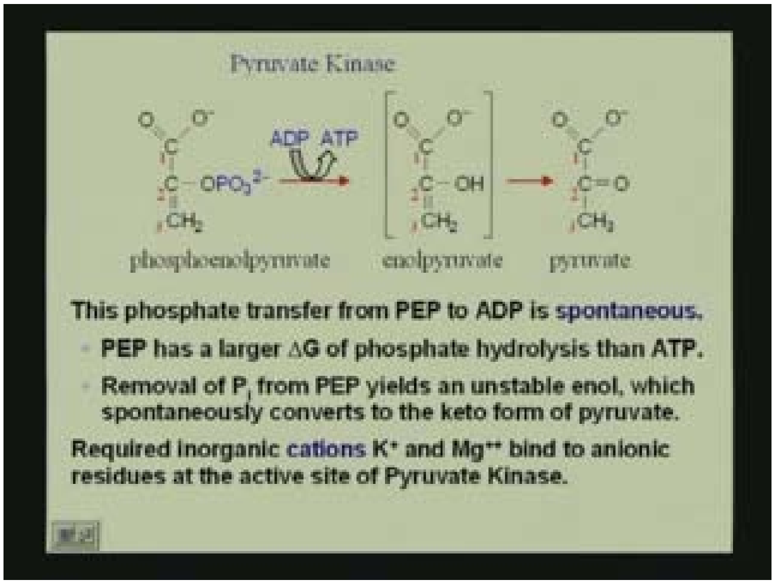

Pyruvate Kinase

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.

Now the formation or the phosphate transfer from PEP. What is PEP? Phosphoenolpyruvate to ADP is a spontaneous reaction. PEP has a larger delta G of phosphate hydrolysis than ATP. What is that mean? It is going to loose the phosphate very easily and ADP will take up this phosphate to form ATP and because you have these anionic residues there are certain cations that bind to the anionic residues of the active site of pyruvate kinase to bring about this particular reaction. So remember that apart from the electron transfer cofactors, apart from the side change that are present since we have carboxylate anions or inolate ions. We have some cations specifically that are used to stabilize the intermediates. So if we lo at the overall glycolysis steps now. We have 10 steps.

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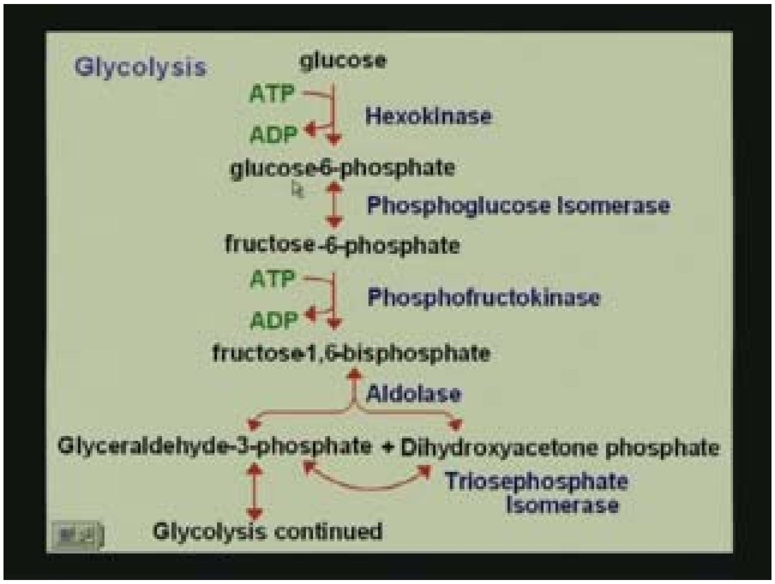

Glycolysis

glucose

ATP

ADP

Hexokinase

glucose6-phosphate

Phosphoglucose Isomerase

fructose-6-phosphate

ATP

ADP

Phosphofructokinase

fructose1,6-bisphosphate

Aldolase

Glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate

Triosephosphate Isomerase

Glycolysis continued

So if we just reiterate we have glucose in the first step going to glucose-6-phosphate. You have to remember that you have to form the pyruvate from the glucose. So the first step is glucose with the help of hexinase and ATP forming glucose-6- phosphate. The next step is an isomerase where we form fructose-6- phosphate; we then need to form fructose 1, 6 bisphosphate which means that we need another kinase and another ATP. This you should remember in this form, so you have glucose, glucose-6- phosphate and since you have to form fructose 1, 6 bisphosphate you have to form fructose- 6- phosphate.

So you need an isomerase that is going to form fructose -6- phosphate from glucose -6- phosphate, with the kinase you have fructose 1, 6 bisphosphate and aldolase acts upon the open ketone form of fructose 1, 6 bisphosphate. That is what I showed you yesterday with the Lysine acting there and we have now a break up into 2, 3 carbon myts. They are dihydroxy acetone phosphate and the glyceraldehydes-3-phosphate. The glyceraldehydes-3- phosphate is going to continue the glycolysis. So what is our glycolysis continued? We have glyceraldehydes-3-phosphate with glyceraldehyde-3-phosphate dehydrogenase with the help of NAD plus and P_i form 1, 3 bisphosphoglycerate.

So you remember in this step when we have to dehydrogenase, you are forming from the glyceraldehydes, you are forming the glycerate. That is important because you have to form finally pyruvate which means you have to have a carboxylic myt. So this is the step where you have that oxidation actually take place from the aldehyde to the acid. So if you have a redox reaction, what is the enzyme that you need? A dehydrogenase or an oxygenase some such enzyme that is going to bring about the redox reaction and for that you need an electron transfer cofactor which in this case is NAD plus.

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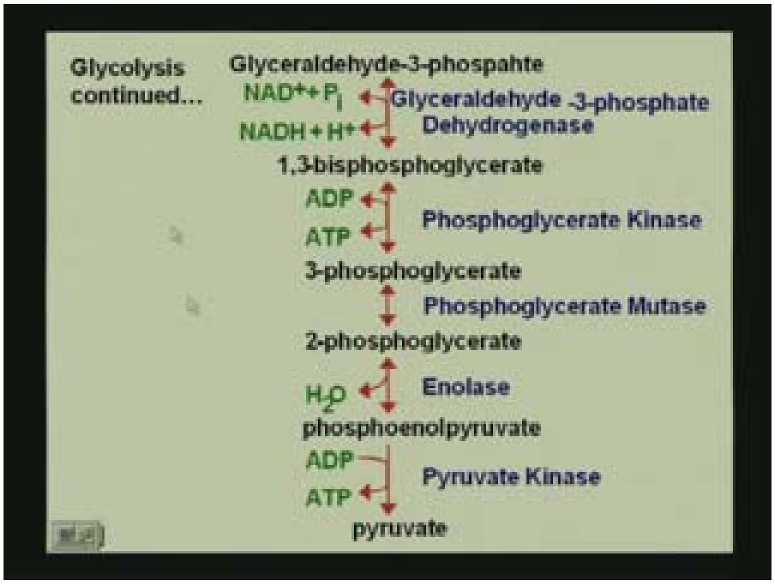

Glycolysis continued...

Glyceraldehyde-3-phospahte

$NAD^+ + P_i$

Glyceraldehyde -3-phosphate Dehydrogenase

$NADH + H^+$

1,3-bisphosphoglycerate

ADP

ATP

Phosphoglycerate Kinase

3-phosphoglycerate

Phosphoglycerate Mutase

2-phosphoglycerate

H_2O

Enolase

phosphoenolpyruvate

ADP

ATP

Pyruvate Kinase

pyruvate

So this is what you would have to remember where you have glyceraldehyde -3-phosphate that takes up NAD plus and a P_i to form 1, 3 bisphosphoglycerate. Then it has to loose the phosphate as simple as that. Now we need the bisphosphoglycerate to oxidized the aldehyde. So for the oxidation of the aldehyde which in turn reduced NAD plus to NADH because this is a redox reaction. What is getting oxidized in this case? Glyceraldehyde is getting oxidized to form glycerate. What is getting reduced? NAD plus is getting reduced to NADH and the enzyme is the dehydrogenase. So we have glyceraldehydes-3- phosphate going to glyceraldehydes-3-phosphate dehydrogenase and in the event it forms 1, 3 bisphosphoglycerate.

Now that you have formed the glycerate, you have to loose the phosphate to form the pyruvate. So the first step in the loss of the one of the phosphates is a phosphoglyceratekinase that is going to loose the phosphate that is attached to the carboxylic first carbon atom of the glycerate. And you have 3 phosphoglycerate formed. After you form 3 phosphoglycerate there is a mutase reaction which shifts the phosphate myt from the third carbon to the second carbon. So you have 3 phosphoglycerate form 2-phosphoglycerate then another enzyme that helps in the dehydration is enolase that results in phosphoenolpyruvate.

So after phosphoenolpyruvate you have the enolic form of pyruvic acid which then forms pyruvate after the loss of the phosphate. And who takes up this phosphate? ADP. So that comprises the whole series of steps where glucose is broken down into pyruvate.

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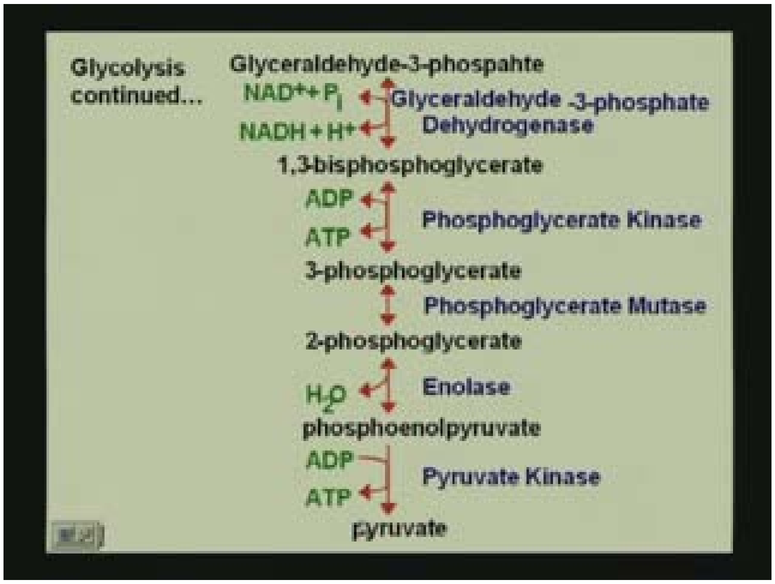

Glycolysis continued...

Glyceraldehyde-3-phospahte
$NAD^{+} + P_i$
Glyceraldehyde -3-phosphate Dehydrogenase
$NADH + H^{+}$
1,3-bisphosphoglycerate
ADP
ATP
Phosphoglycerate Kinase
3-phosphoglycerate
Phosphoglycerate Mutase
2-phosphoglycerate
H_2O
Enolase
phosphoenolpyruvate
ADP
ATP
Pyruvate Kinase
pyruvate

Now what we have to see is we have to do a balance of energy, we have to see how many ATP's are taken up, how many ATP's are produced and to see whether the actual breakdown of glucose is giving us any energy at all. There is one thing that we have to remember that per glucose there are 2 glyceraldehyde 3 phosphates because what is the previous step? We have two of these and in the triosephosphate isomerase we know that the equilibrium is shifted to this side because this is being consumed in the further steps. So our balance sheet for the phosphate bonds of ATP, how many ATP bonds are broken? How many are broken? This is our whole series, we have one broken when we form glucose to glucose -6- phosphate another broken when we form fructose 1, 6 bisphosphate from fructose -6- phosphate [Refer Slide Time: 27:20].

So our answer for the first question is 2. How many phosphate bonds of ATP are produced? How many are produced? Is it 2 from every molecule of glucose 4, why? Because we have two of these, 1 glyceraldehyde 3 phosphate is going to create 2 ATP's but in our previous step what do we have? We have two of these myts because we have broken down the 6 membered ring in 6 carbons into 3 carbon molecules. So because of the 2, that is why I specifically mentioned that there are 2 G3P that is glyceraldehyde 3 phosphates per glucose. So we have 4, so how many bonds of ATP are produced? 4. And the net production per glucose is because we are utilizing 2 and we are creating 4, so eventually we get some energy after we break down a molecule of glucose.

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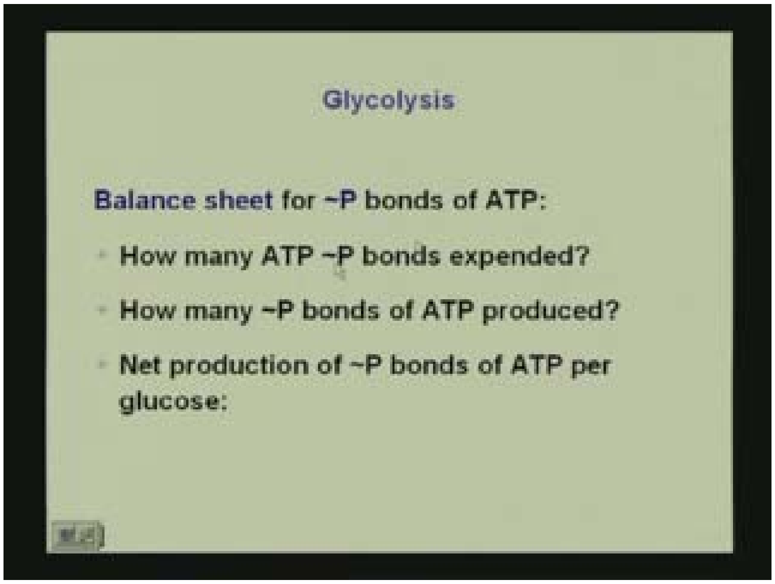

Glycolysis

Balance sheet for ~P bonds of ATP:

- How many ATP ~P bonds expended?
- How many ~P bonds of ATP produced?
- Net production of ~P bonds of ATP per glucose:

So this [Refer Slide Time: 28:37] what we have? We have 2 ATP expended, 4 ATP produced that is 2 from each of the two 3 carbon fragments from glucose and the net production there is 2 phosphate bonds of ATP or 2 ATP rather per glucose. So our overall step is going to be glucose plus 2 NAD plus, where did we use this NAD plus? We use just one here, two here because we have again 2 of G3P. So this is where we are using two of them. Then 2 ADP and 2 P_i? Where was P_i used again? In the same step where you had NAD plus, we used the P_i to form the phospo and what is finally formed? 2 pyruvate from 1 glucose two 3 carbon myts 2 NADH plus 2 ATP. So that is our total pathway.

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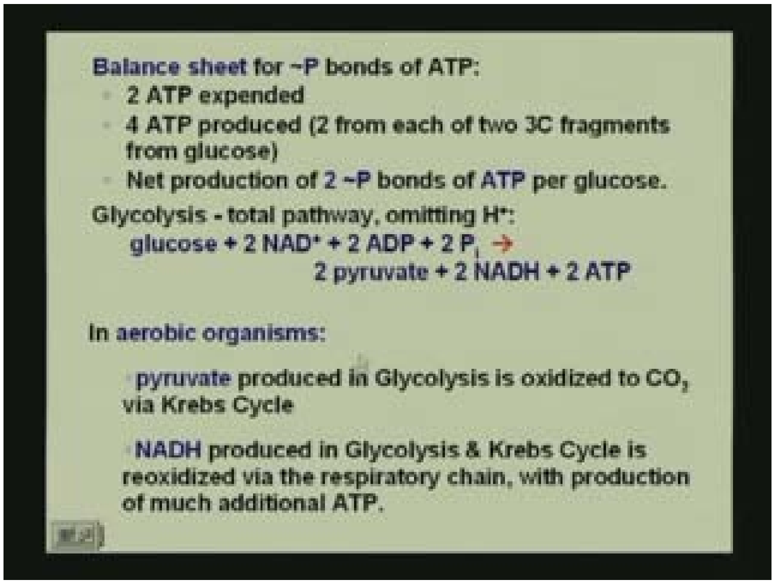

Balance sheet for ~P bonds of ATP:

- 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^+:

$$\text{glucose} + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ ATP$$

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.

Now in aerobic organisms you have pyruvate that is produced in glycolysis oxidized to carbon dioxide that is what we are going to see when we do the kerbs cycle. This is also known as the TCA cycle and the NADH, you have NADH produced here, this NADH that is produced in glycolysis and also in the kerbs cycle is reoxidized. What is it means to be reoxidizing NADH? Means you have to get back NAD plus because you need NAD plus for the glycolysis, now it is already NADH. The only way you can get back is by the respiratory chain that is the oxidative phophorylation which we studied with production of a lot of additional ATP which we studied with the proton pump. There was mainly FADH and NADH that was used there [Refer Slide Time: 30:50].

Now another thing that we have to look at is anaerobic organisms, when we have aerobic organisms we have the formation of pyruvate but in this case what happens there is the formation of lactate instead of pyruvate where for example even in our muscles where there is a lack of oxygen at times pyruvate is formed in to lactate. Now in anaerobic organisms, what is an anaerobic organism? They function without the presence of oxygen.

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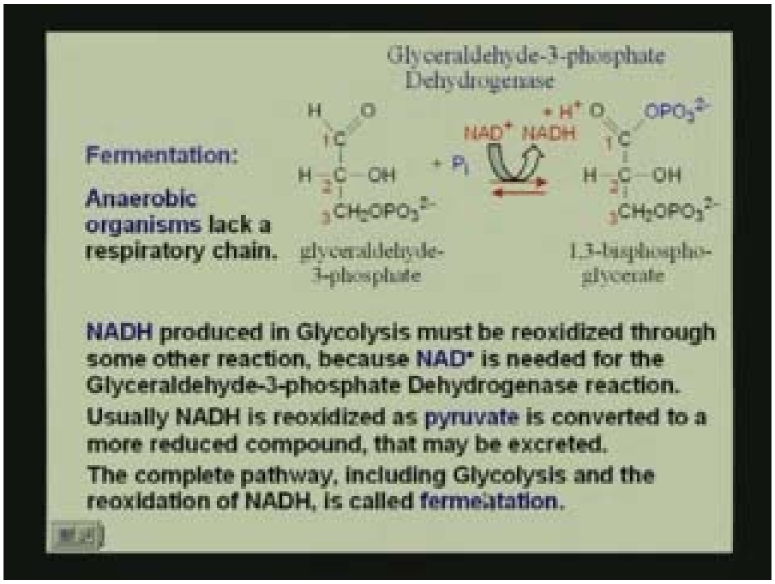

Fermentation:

Anaerobic organisms lack a respiratory chain.

NADH produced in Glycolysis must be reoxidized 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, that may be excreted.

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

They lack a respiratory chain in the absense but in the formation of glyceraldehyde 3 phosphate two 1, 3 bis phosphoglycerate where we have the NAD plus and the P_i. This is the one of the reactions in glycolysis. Now the NADH produced in glycolysis must be reoxidized through some other reaction because it is needed for this reaction and the NADH is reoxidized as pyruvate is converted to a more reduced compound later on that may excreted. This is one of the cases and the complete pathway including glycolysis and the reoxidation is actually called fermentation, we will see that. What we have here is now we created pyruvate from glucose, that was our final step in our glycolytic cycle. Now we can reform NAD plus, remember what did we take in the bis phosphoglycerate formation. We took NAD plus and made NADH out of it.

Now lactate dehydrogenase, it actually reduces the keto in pyruvate and in the event it oxidizes NADH to NAD plus, again it is a redox reaction. So the enzyme is going to be a dehydrogenase. Now for example this is what happens in skeletal muscles that ferment glucose to lactate during exercise when aerobic metabolism cannot keep up with energy requirement. So what happens is this pyruvate is further, what happens? There is a reduction of the ketone in the pyruvate and this reduction will eventually do what? Oxidize NADH to NAD plus, so the lactate released to the blood may be taken up by other tissues or by muscle after exercise and converted via the reversible lactate dehydrogenase back to pyruvate to get into krebs cycle.

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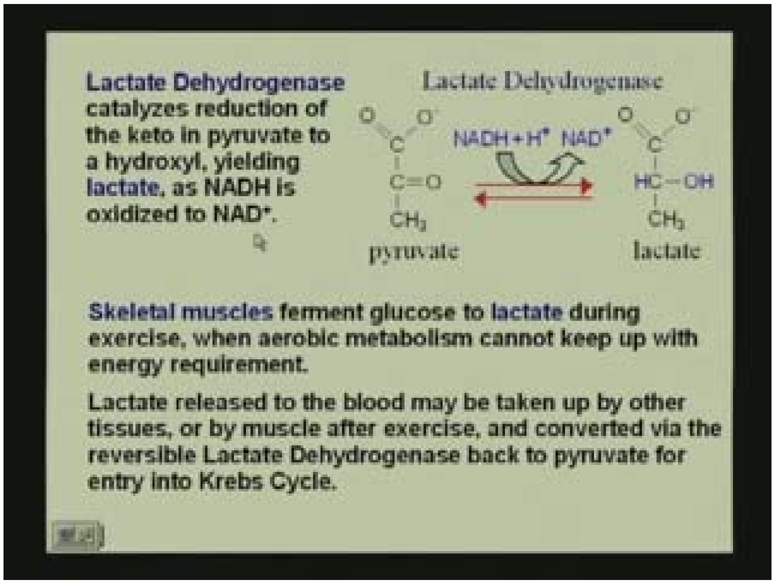

Lactate Dehydrogenase catalyzes reduction of the keto in pyruvate to a hydroxyl, yielding **lactate**, as NADH is oxidized to NAD$^+$.

Skeletal muscles ferment glucose to **lactate** during exercise, when aerobic metabolism cannot keep up with energy requirement.

Lactate released to the blood may be taken up by other tissues, or by muscle after exercise, and converted via the reversible Lactate Dehydrogenase back to pyruvate for entry into Krebs Cycle.

So this is sort of a stop gap situation where we would have in the lack of aerobic metabolism that would lead to the lactate formation where there is not enough oxygen present. Now some anaerobic organisms metabolize pyruvate to ethanol. This process you have learnt fermentation like yeast this is a reaction that yeast often do where you have industrial chemistry where you learn about a lot of this, where we have NADH go to NAD plus by alcohol dehydrogenase.

In the first step the pyruvate looses carbon dioxide to form acetaldehyde that is one step of the reaction. This acetaldehyde then in the presence of alcohol dehydrogenase forms ethanol and in the event NADH is oxidized to NAD plus. So they metabolize pyruvate to ethanol which is actually a waste product. This is [Refer Slide Time: 34:58] a process in fermentation, so animal cells also contain alcohol dehydrogenase which oxidizes ethanol to acetaldehyde.

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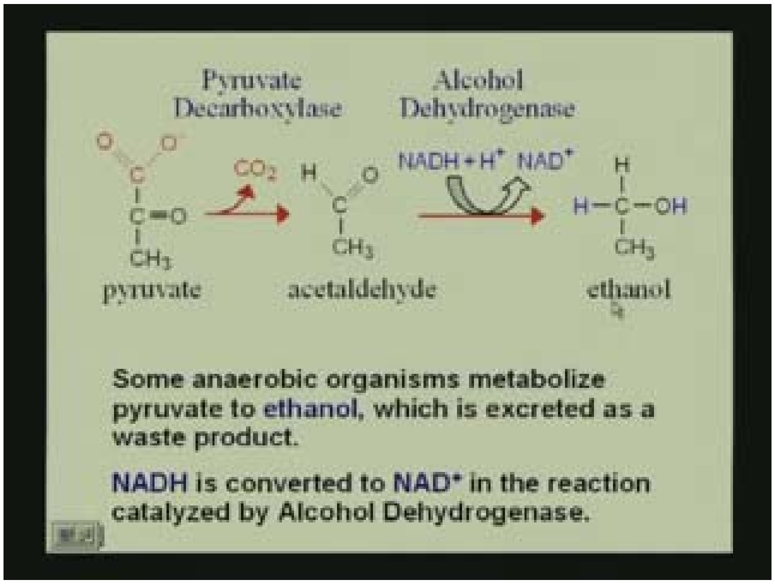

Some anaerobic organisms metabolize pyruvate to ethanol, which is excreted as a waste product.

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

So we have ethanol then we have NAD plus that is getting reduced to NADH and what is it doing? It is oxidizing ethanol to acetaldehyde and then further oxidation is also possible to acetate where you have NAD plus going to NADH plus H plus. So what are you doing in this case? You are reducing NAD plus in the event oxidizing acetaldehyde to acetate. In fact the animal cells that contain alcohol dehydrogenase in the forming of acetaldehyde, this is what results in the hang over that you get from alcohol consumption.

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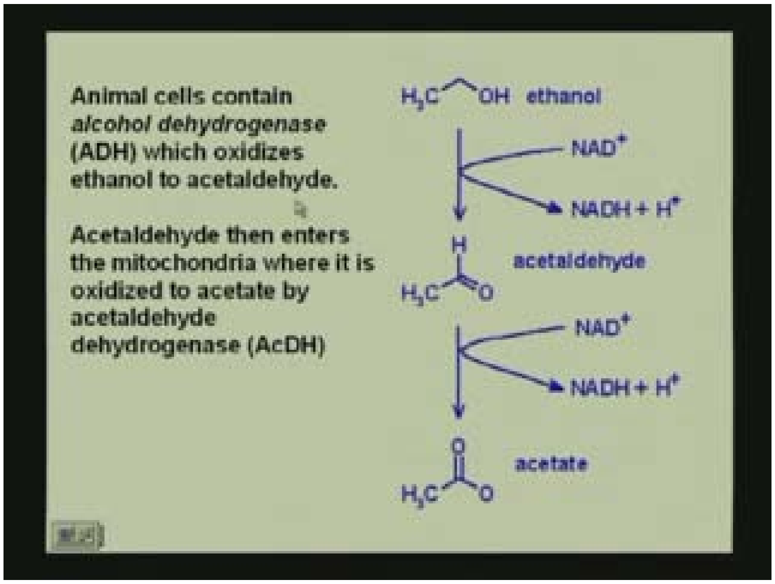
Animal cells contain *alcohol dehydrogenase* (ADH) which oxidizes ethanol to acetaldehyde.

Acetaldehyde then enters the mitochondria where it is oxidized to acetate by acetaldehyde dehydrogenase (AcDH)

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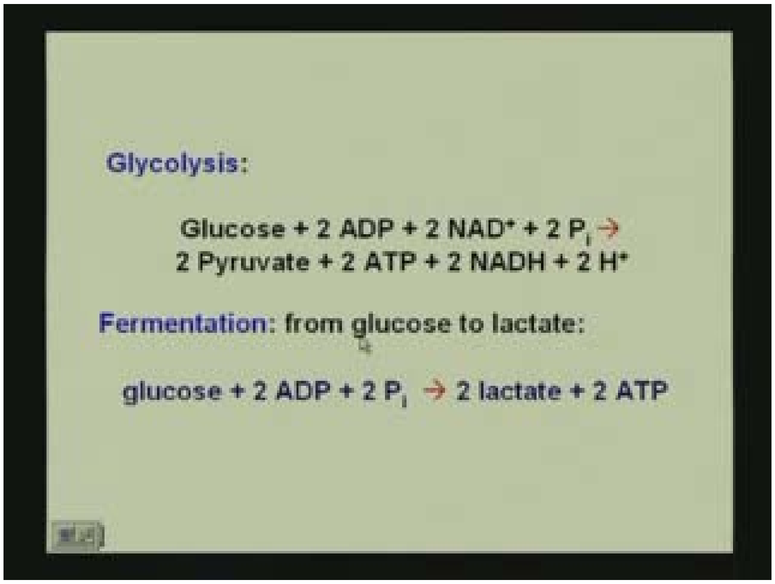

Glycolysis:

$\text{Glucose} + 2\,\text{ADP} + 2\,\text{NAD}^+ + 2\,\text{P}_i \rightarrow$
$2\,\text{Pyruvate} + 2\,\text{ATP} + 2\,\text{NADH} + 2\,\text{H}^+$

Fermentation: from glucose to lactate:

$\text{glucose} + 2\,\text{ADP} + 2\,\text{P}_i \rightarrow 2\,\text{lactate} + 2\,\text{ATP}$

You hear of hangovers from alcohol consumption, what happens is this ethanol gets oxidized to acetaldehyde in the body by this particular enzyme alcohol dehydrogenase and this acetaldehyde actually reacts with certain proteins to give you that numb feeling. So it is all bio chemistry that goes on. So this [Refer Slide Time: 36:15] our final step of glycolysis where we have our glucose 2 ADP 2 NAD plus going to 2 phosphate plus 2 phosphate that forms 2 pyruvate 2 ATP 2 NADH plus 2 H plus.

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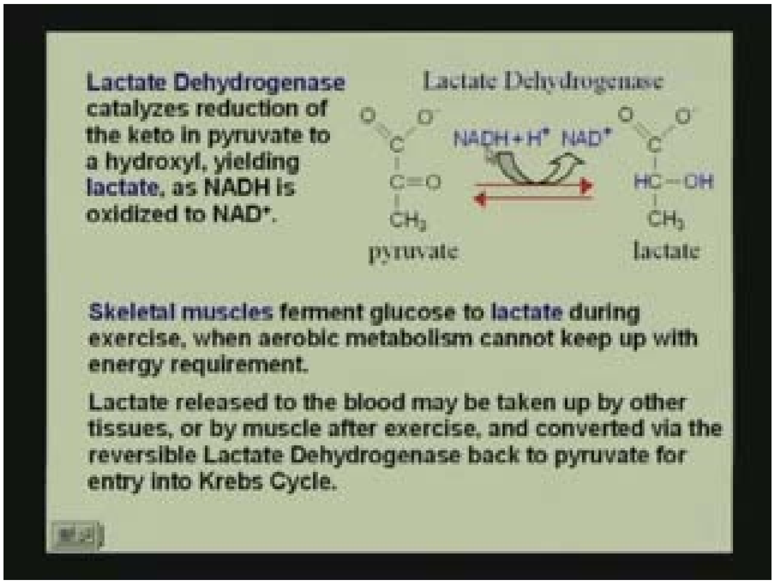

Lactate Dehydrogenase catalyzes reduction of the keto in pyruvate to a hydroxyl, yielding **lactate**, as NADH is oxidized to NAD^+.

Skeletal muscles ferment glucose to **lactate** during exercise, when aerobic metabolism cannot keep up with energy requirement.

Lactate released to the blood may be taken up by other tissues, or by muscle after exercise, and converted via the reversible Lactate Dehydrogenase back to pyruvate for entry into Krebs Cycle.

In the fermentation instead of pyruvate we are going to get lactate, so we have the glucose that with 2 ADP plus 2 P_i will give us 2 lactate plus 2 ATP because remember when we are forming the pyruvate to the lactate, you just go back a step [Refer Slide Time: 36:55] this NADH, NAD plus does not come into the picture because if we look at this reaction, we have the NAD plus and the P_i here forming NADH but that is not the NADH is utilized in the pyruvate reaction to lactate. So we do not have NAD plus NADH feature in the fermentation where we are creating lactate from glucose because that pyruvate to lactate conversion requires NAD plus NADH.

So if we now look at all the enzymes that were involved there are 10 enzymes here for the 10 steps in glycolysis. Hexinase, isomerase, so now you should be able to tell me exactly what each of these steps are doing. Hexinase creates glucose 6 phosphate from glucose, isomerase creates fructose 6 phosphate from glucose 6 phosphate, phophofructinase creates the fructose 1, 6 bis phosphate from the fructose 6 phosphate, aldolase breaks it up into dihydroxy acetone phosphate and glyceraldehyde 3 phosphate.

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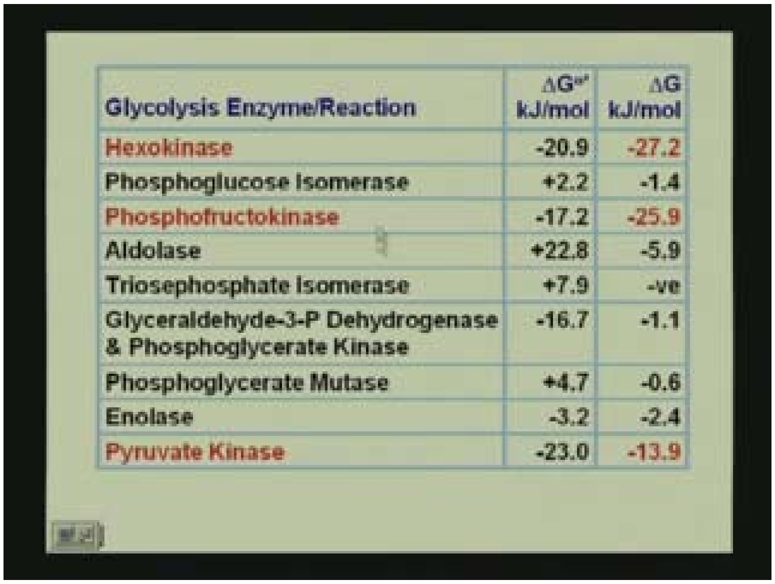

Glycolysis Enzyme/Reaction	$\Delta G^{o\prime}$ kJ/mol	ΔG kJ/mol
Hexokinase	-20.9	-27.2
Phosphoglucose Isomerase	+2.2	-1.4
Phosphofructokinase	-17.2	-25.9
Aldolase	+22.8	-5.9
Triosephosphate Isomerase	+7.9	-ve
Glyceraldehyde-3-P Dehydrogenase & Phosphoglycerate Kinase	-16.7	-1.1
Phosphoglycerate Mutase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

The isomerase inter converts dihydroxy acetone phosphate and glyceraldehyde 3 phosphate then we have glyceraldehyde 3 phosphate dehydrogenase then we have phosphoglycerate kinase that is going to create the best phosphoglycerate. The best phosphoglycerate is then has a mutase acting on it where it creates from three phosphoglycerate it goes to 2 phosphoglycerate then we have the enolase that creates the phosphoenol pyruvate that then looses because when you have the enolase you have to have an enol present there, so you have phopho enol pyruvate that phosphoenol pyruvate looses the phosphorus by pyruvate kinase to from pyruvate. So these are all the steps of glycolysis and if we now look at the specific free energy changes involved in the steps we have a delta G zero prime.

A prime means a biological standard where the temperature is taken as 37 degree centigrade instead of 25 degree centigrade because you are looking at the free energy changes that are happening in the body when the reactions are taking place. In each of the kinase reaction this is just the delta G with the concentrations that we have, this is delta G zero. So how do we calculate delta G? Using delta G zero and you need the concentrations of the reactants and the products, that is all you need. How do you do? You calculate an equilibrium constant for a delta G zero process and then you have the reaction quotient that is going to depend upon the concentration of the reactants and the products that you have and you can calculate the delta G associated with the process provided you know the delta G zero.

So considering that the concentrations of products and the reactants can be calculated or can be obtained, we get a delta G associated with these enzymes, 10 of these enzymes and if you notice the kinase enzymes are the ones that are most spontaneous. They have high kilo joule per mole value and the reason why they have this is because this is a highly spontaneous reaction where it is going to loose the phosphate. These are the ones that use couple reactions with the ATP that provides the energy actually to give you the product.

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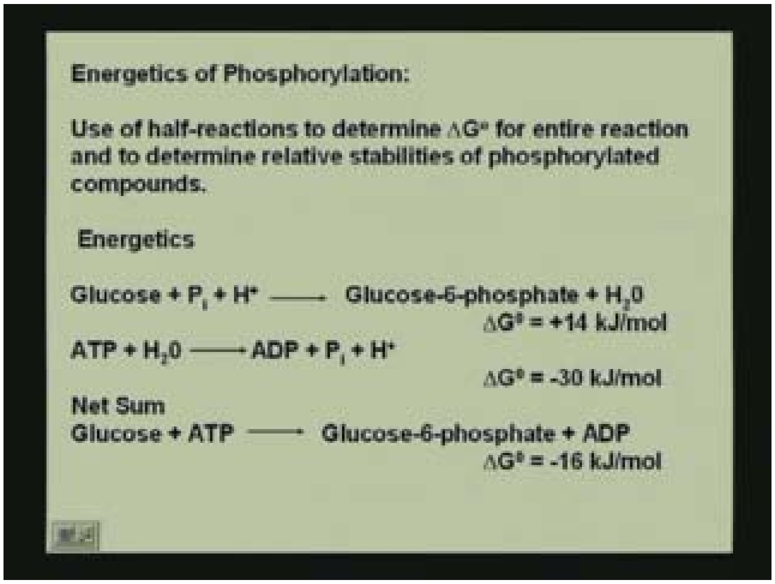

Energetics of Phosphorylation:

Use of half-reactions to determine ΔG^{o} for entire reaction and to determine relative stabilities of phosphorylated compounds.

Energetics

$$\text{Glucose} + P_i + H^+ \longrightarrow \text{Glucose-6-phosphate} + H_2O \qquad \Delta G^0 = +14 \text{ kJ/mol}$$

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i + H^+ \qquad \Delta G^0 = -30 \text{ kJ/mol}$$

Net Sum

$$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose-6-phosphate} + \text{ADP} \qquad \Delta G^0 = -16 \text{ kJ/mol}$$

So what we have is if we look now at the energetics of phophorylation, we are looking at the first step hexokinase, the glucose actually needs the phosphate to become glucose 6 phosphate, the delta G zero associated with that is actually plus 14 kilo joules per mole. So there is no way it is going to happen by itself but you have a couple reaction that is ATP, the hydrolysis of ATP the high energy phosphate bond that is actually going to break ATP into ADP plus P_i. This P_i is going to be supplied to glucose, this free energy change is minus 30 kilojoules per mole. So we have this couple reaction so we use the half reaction to determine the delta G zero for the whole reactions.

And we have therefore an over an overall reaction that is going to be glucose plus ATP giving us glucose 6 phosphate plus ADP with a favorable free energy standard free energy change that we have. Now depending on the concentrations of glucose 6 phosphate that you have and the concentrations of glucose and the relative amounts of ATP and ADP that you have, the free energy change of the reaction is going to change. Because what is that reaction that we have? If we look at a delta G zero value, now what you can have is now for each of these you can have an E zero also associated with it because remember you have a redox reaction taking place.

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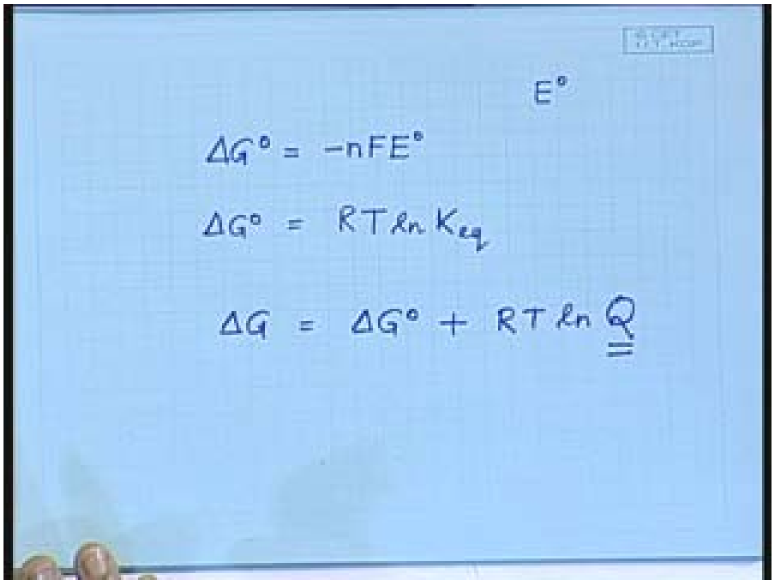

E°

$$\Delta G^\circ = -nFE^\circ$$

$$\Delta G^\circ = RT \ln K_{eq}$$

$$\Delta G = \Delta G^\circ + RT \ln \underline{\underline{Q}}$$

In some cases you would have a redox reaction taking place where you would have this minus n FE. From this you can determine the equilibrium constant. What is the equation? Equilibrium, then you can have a delta G. What is that? That is delta G zero plus RT ln Q. What is this Q? This Q is your reaction quotient. What is the reaction quotient? It is the ratio of the concentration of the products to the reactants. So based on that you can find the delta G associated with this. Now if we look at this first reaction that we have here the fact that glucose 6 phosphate concentration is low. In this case what is the Q going to be? It is going to be glucose 6 phosphate ADP divided by glucose ATP concentration that is what Q is going to be.

Now if you have a high concentration of products that could compensate more than the delta G zero value negative that you have here and make an overall delta G of the reaction positive. What am I talking about? I am talking about this factor, this is negative but the ratio of the product to the reactants is important here. You cannot have too higher concentration of the products because that will over compensate for the negative delta G zero that you have here making this non spontaneous. This is what happens in most biological reactions, the product concentrations are pretty low making the reaction spontaneous because your delta G zero of the coupled reaction is negative.

So the concentration of glucose 6 phosphate in this particular reaction is actually low and the overall Gibbs free energy that you get is minus 27 kilo joules per mole. So what you can do with this value is actually determined what the ratio of the products to the reactants are, you know what delta G zero is, you know what delta G is. So you can find the ratio of the products to the reactants and when you are considering, if it is not mentioned a delta G zero prime value means you use a temperature of 37 degree centigrade. So that would be how you would actually calculate the energetics of every step of glycolysis.

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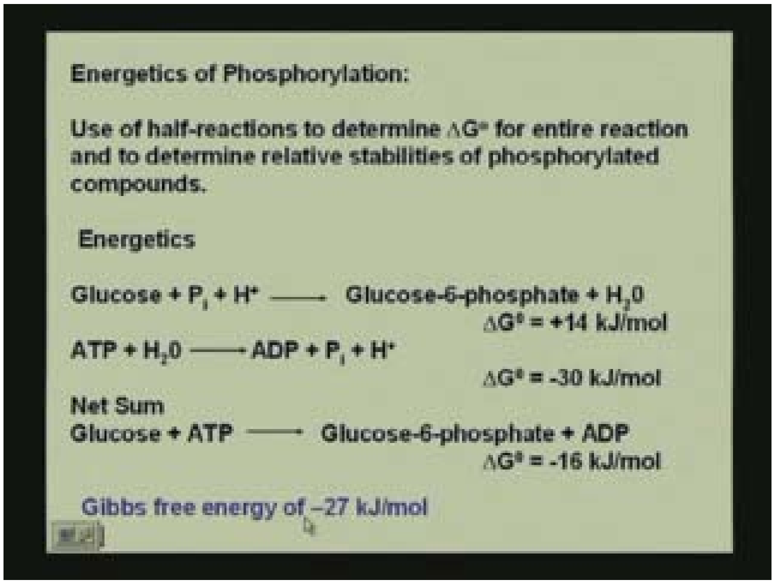

Energetics of Phosphorylation:

Use of half-reactions to determine ΔG^{o} for entire reaction and to determine relative stabilities of phosphorylated compounds.

Energetics

$\text{Glucose} + P_i + H^+ \longrightarrow \text{Glucose-6-phosphate} + H_20$

$\Delta G^{o} = +14$ kJ/mol

$\text{ATP} + H_20 \longrightarrow \text{ADP} + P_i + H^+$

$\Delta G^{o} = -30$ kJ/mol

Net Sum

$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose-6-phosphate} + \text{ADP}$

$\Delta G^{o} = -16$ kJ/mol

Gibbs free energy of –27 kJ/mol

We can calculate the energetics associated with every step of glycolysis and find an overall energetics associated with this. Now the spontaneous reaction that we did see that was with this particular case this enzyme is hexokinase and the other ones that I showed you were phophofructinase and pyruvate kinase. These are the 3 enzymes that actually catalyze spontaneous reactions and the control of these enzymes actually determines the rate of glycolysis because each of these particular reactions if you go back and look at the glycolysis pathway, it is these three reactions that are irreversible.

So once glucose forms glucose 6 phosphate it is trapped in the cell. So the control of these enzymes actually determines the rate of the glycolysis pathway. So there are two types of control, we have local control and global control. Local control means that it involves dependence of enzyme catalyzed reactions on concentrations of the pathway substrates or intermediates within a cell. So what is this local control? This is exactly what I was speaking about in the last slide where we have the delta G values associated with the amount of product and reactant that you have.

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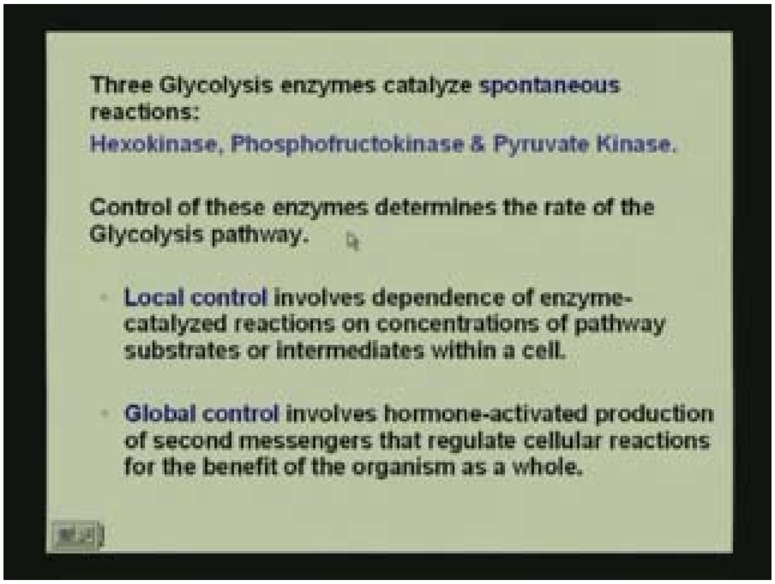

Three Glycolysis enzymes catalyze spontaneous reactions:
Hexokinase, Phosphofructokinase & Pyruvate Kinase.

Control of these enzymes determines the rate of the Glycolysis pathway.

- **Local control** involves dependence of enzyme-catalyzed reactions on concentrations of pathway substrates or intermediates within a cell.
- **Global control** involves hormone-activated production of second messengers that regulate cellular reactions for the benefit of the organism as a whole.

So if my product concentration gets too high, what is going to happen? My reaction will be non spontaneous, so that is where you would have local control. If glucose 6 phosphate has too higher concentration, the delta G associated with the expression that we have written here will make this non spontaneous. So this is regulated by the amount glucose 6 phosphate that is formed because remember I mentioned in the last class that glucose 6 phosphate actually inhibits hexokinase. So what is going to happen? If it inhibits hexokinase then this enzyme cannot act on another glucose molecule which means that the concentration of glucose 6 phosphate is getting too high, making this Q too large which is offsetting the delta G zero preventing a reaction from taking place. You do not want that to happen. If you want the glycolysis reaction to go forward then you have to have this regulation. You do not want too much of glucose to be broken down, that is where the regulation takes place.

So we have local control which involves the dependence of enzyme catalyzed reactions on concentrations of pathway substrates or intermediates within a cell. You now realize what this means that the concentrations of the intermediates, the concentrations of the products and the substrates are extremely important in determining the dependence of these catalyzed reactions. Then you have global control that actually involves hormone activated production of second messengers, these are certain messengers that are there in the body that actually regulate the cellular reactions for the benefit of the organism as a whole. So this is like a more global approach where it could work on the cell itself preventing the cell from acting or preventing certain reactions taking place all together. But at the local level the concentration dependence are due to the variations in the product and reactant. Now let us look at one such example, this is one step of the glycolytic pathway.

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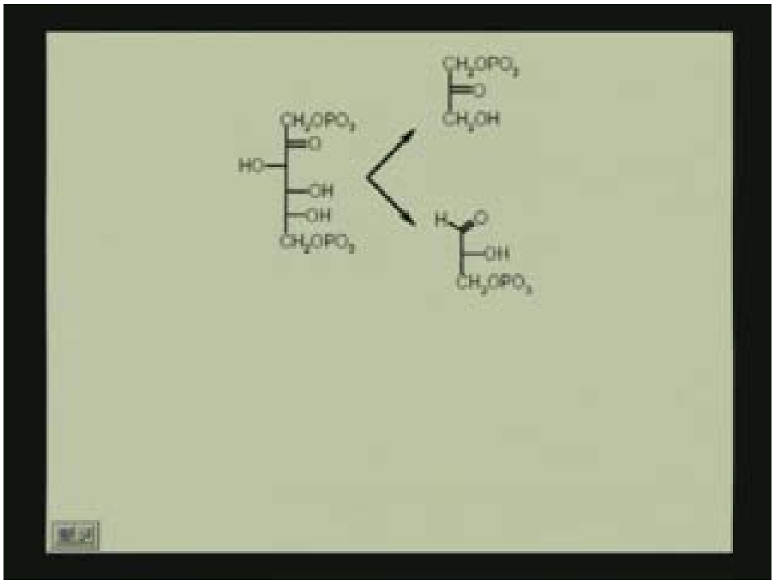

If you look at this, you see it has 2 phosphates attached carbon number 1 and carbon number 6 you have a ketose. So this is fructose 1, 6 bisphosphate, it has broken down into dihydroxy acetone phosphate and glyceraldehyde -3- phosphate. So the enzyme that has acted here is aldolase in a reverse aldol reaction. So this is what you should be able to recognize, this is the key step in the glycolysis. Why? Because it is actually breaking down the glucose, previously all the steps that led to fructose 1, 6 bisphosphate still have the 6 carbon atoms in it. This step, the aldolase step is the one that you are breaking the 6 carbon unit into 3 carbon units. Now we have a delta G_0 of this particular reaction having a value of plus 23.8 kilo joules per mole.

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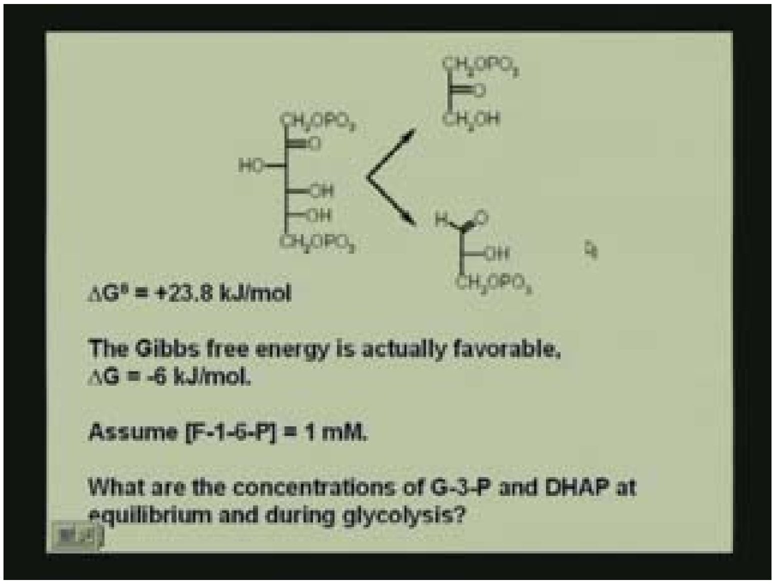

$\Delta G^0 = +23.8$ kJ/mol

The Gibbs free energy is actually favorable, $\Delta G = -6$ kJ/mol.

Assume [F-1-6-P] = 1 mM.

What are the concentrations of G-3-P and DHAP at equilibrium and during glycolysis?

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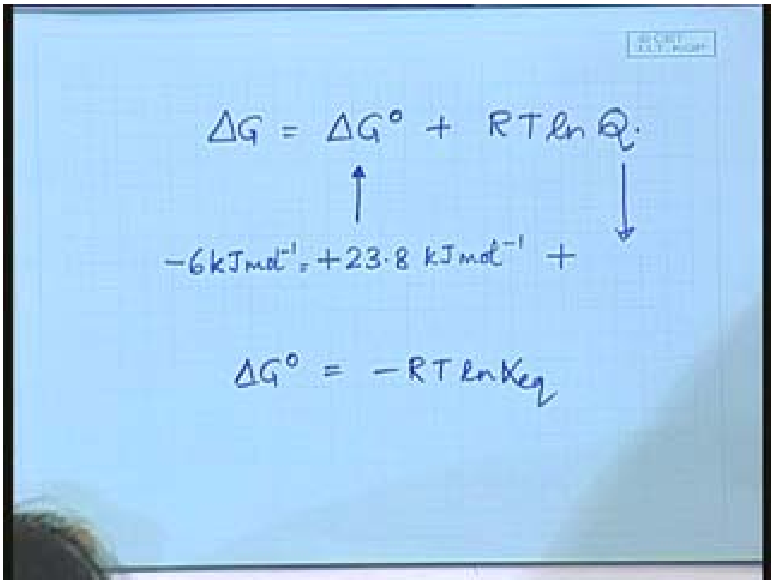


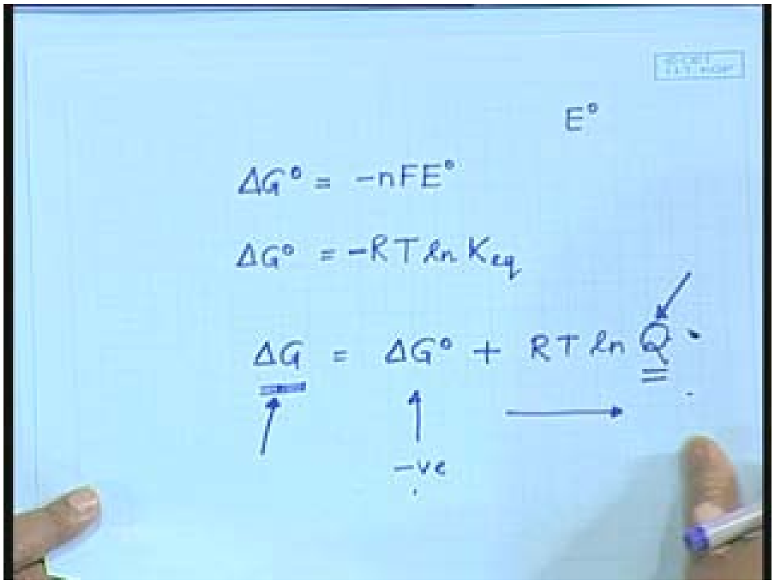
$$\Delta G = \Delta G^\circ + RT \ln Q.$$

$$-6\,kJ\,mol^{-1} = +23.8\,kJ\,mol^{-1} +$$

$$\Delta G^\circ = -RT \ln K_{eq}$$

The Gibbs free energy is actually favorable. What is that mean I have a delta G lets just write this down clearly. So I have a delta G and I have delta G zero plus RT ln Q, this value is plus 23.8 kilo joules per mole. This value is minus 6 kilojoules per mole, so where is the regulation coming from? It has to come from Q. So what we can do is we can actually calculate assuming that fructose 1, 6 bisphosphate is 1 millimolar. I have these in equal concentration because it is breaking down, it forming from this. So I can actually find out the concentrations of glyceraldehyde 3 phosphate and dihydroxy acetone phosphate at equilibrium. How can I do that at equilibrium? It will just be at the equilibrium constant that I get, from the delta G_0 value.

[Refer Slide Time: 53:05]



E°

$$\Delta G^\circ = -nFE^\circ$$

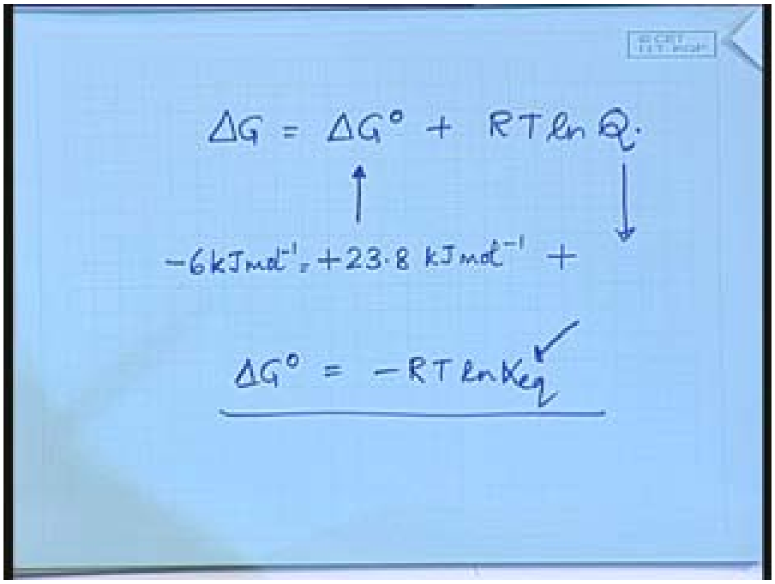
$$\Delta G^\circ = -RT \ln K_{eq}$$

$$\Delta G = \Delta G^\circ + RT \ln Q.$$

$-ve$

What do I get from the delta G zero value? I forgot to write a minus in the first one. So I can get this [Refer Slide Time: 53:42] because I know what my delta G zero. So I can get the ratio of the products to the reactants based on considering 1 millimolar concentration of fructose 1, 6 bisphosphate and I can also find the same ratio during glycolysis where my reaction is actually favorable.

[Refer Slide Time: 53:05]



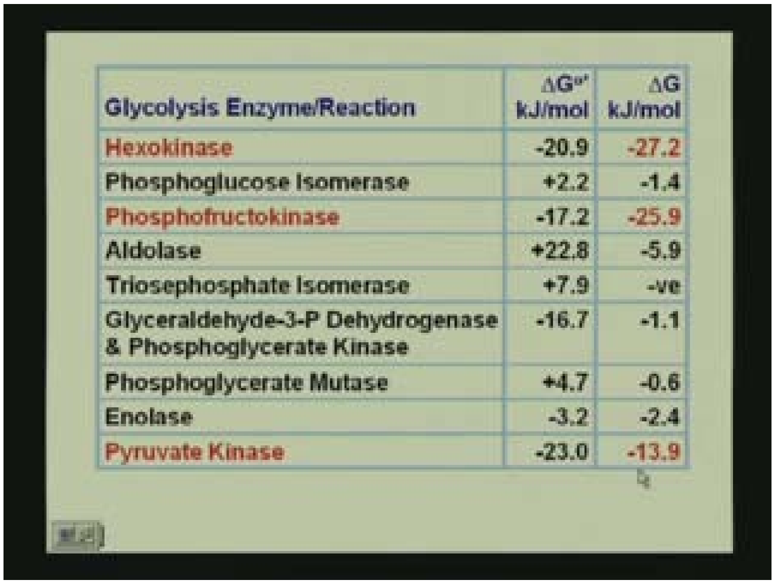
$$\Delta G = \underset{\uparrow}{\Delta G^{\circ}} + RT \ln \underset{\downarrow}{Q}.$$

$$-6\,\mathrm{kJ\,mol^{-1}} = +23.8\,\mathrm{kJ\,mol^{-1}} +$$

$$\underline{\Delta G^{\circ} = -RT \ln K_{eq}}$$

So this whole idea gives you the energetics that are associated with the each of these steps. Each of these steps has associated with this energy [Refer Slide Time: 53:45].

[Refer Slide Time: 53:44]



Glycolysis Enzyme/Reaction	$\Delta G^{o\prime}$ kJ/mol	ΔG kJ/mol
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Phosphofructokinase	-17.2	-25.9
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Triosephosphate Isomerase	+7.9	-ve
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

So each of these energy values can tell me what is actually happening and what delta G zero reaction actually have the equilibrium. So this completes our discussion on glycolysis where we have actually broken down glucose to form pyruvate. Our next step will now be to see how there is further degradation of pyruvate in the tricarboxylic acid cycle where finally carbon dioxide and water will be formed. We will do that in our next class. Thank you.