Biochemical Engineering
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Module No. # 01
Lecture No. # 18
Bioenergetics and Glycolysis

My today's topic of teaching is Bioenergetics and Glycolysis. Now, in this class we will learn how the energy is getting changed inside the cell.

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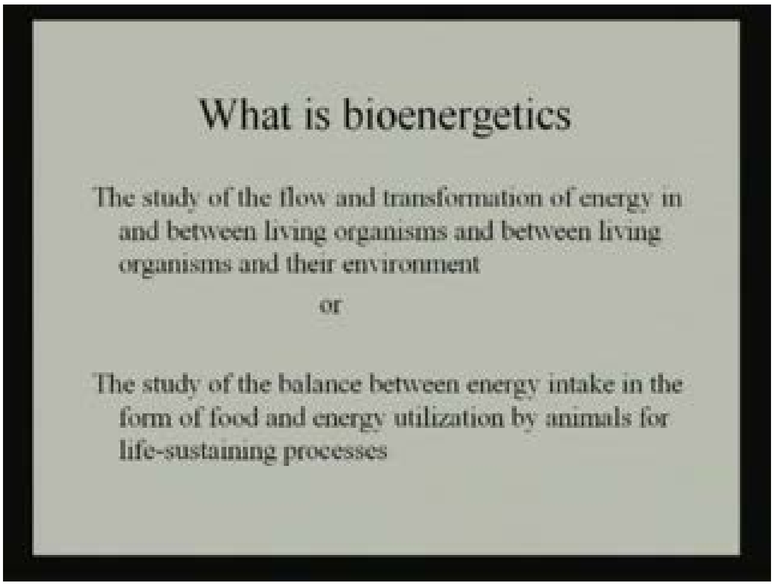

What is bioenergetics

The study of the flow and transformation of energy in and between living organisms and between living organisms and their environment

or

The study of the balance between energy intake in the form of food and energy utilization by animals for life-sustaining processes

Now, when we are taking about any living system, then we have already learned that, there are different activities, which are going on inside the cell and those activities when it is taking place, it is the energy driven process. Now, when we are studying the bioenergetics, it is the study of flow and transformation of energy in and between the living organisms and between the living organisms and their environment that means, it may be within the cell or it may be the cells and their surroundings. The study of the balance between energy intake in the form of food and energy utilisation by the animals for life-sustaining processes.

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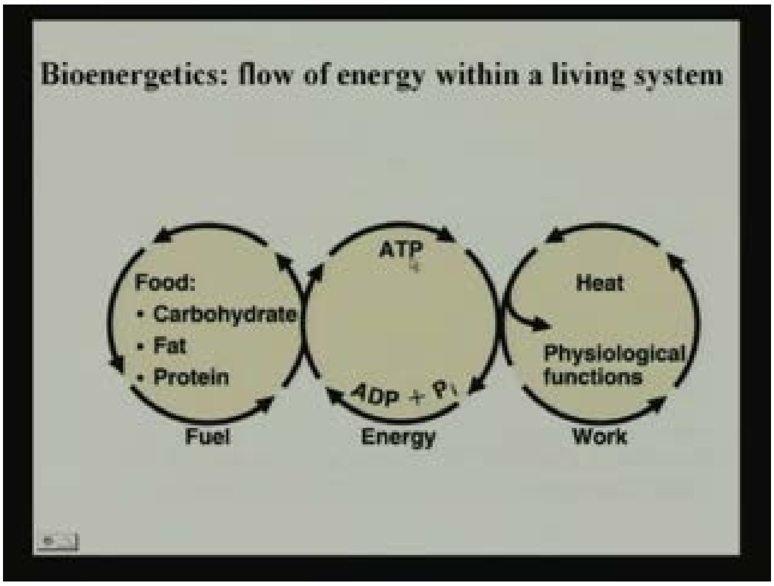

Bioenergetics: flow of energy within a living system

Food:
- Carbohydrate
- Fat
- Protein

Fuel

ATP

ADP + Pi

Energy

Heat

Physiological functions

Work

Now, when we are talking about the different activities in any living system obviously, we are talking about the food, we are taking food at a as a as a source of energy. Now, when we are taking this food, we are taking some of the macromolecules, what I have already talked and I have already told you about this carbohydrate, fats, and proteins and so on, along with these macromolecules, some of the micro molecules are also being assimilated within the living system.

Now, when this macromolecules along with this micro molecules are taken ingested by the body then it is getting assimilated, it is getting absorbed, it it is getting digested and then it forms the energy, it produce energy. And in any living system, when we are talking about the energy, we mainly talk about the ATP energy, now ATP is one of the major important forms of energy.

Now, ATP is catalysing different activities, different such reaction and when these reactions are going on inside any living cell it produce, some amount of heat. Now, this obviously, when it is this heat is being produce, the different functions physiological functions are also simultaneously going on and we are calling it as the cell is performing some activity or work.

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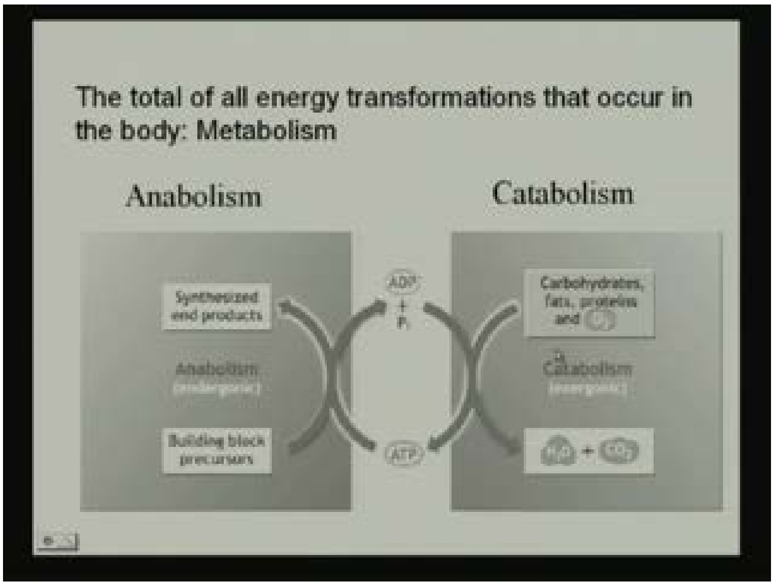

The total of all energy transformations that occur in the body: Metabolism

Now, when we are talking about the transformation of energy from one form to another, we cannot we cannot divide of any some the terminology which is called as metabolic activities. Now, what is the metabolism, metabolism is an universal process, it is complex and irreversible process, now the entire metabolic activities of any living cell can be of two types, either it is the anabolic process or it is catabolic process, now what is the anabolic process, when the cell is synthesising something it is called anabolism.

Now, see here the building block of precursors are there within the cell and some synthesis is going on it is the endergonic process and some product is being synthesized by the cell, by system, by the cell. And when it is the catabolic process the macromolecules which are present within the cell, say in the form of carbohydrate, fats, proteins and so on; this macromolecules are getting is broken down into micro molecules, that is macromolecules are getting chopped into micro molecules and this way the different functions or different activities are being performed by the cell.

Now, photosynthesis is one of the example of the anabolic process, and respiration is another example, which is going on inside the cell is the catabolic process.

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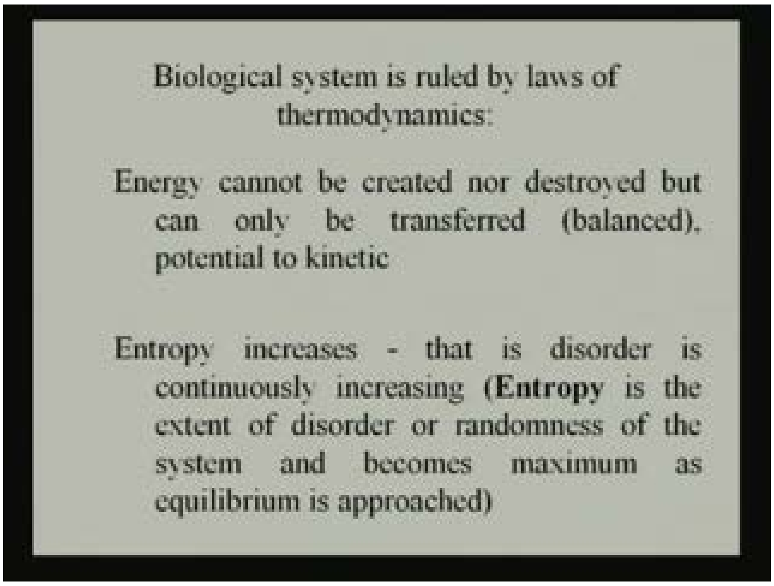

Biological system is ruled by laws of thermodynamics:

Energy cannot be created nor destroyed but can only be transferred (balanced), potential to kinetic

Entropy increases - that is disorder is continuously increasing (**Entropy** is the extent of disorder or randomness of the system and becomes maximum as equilibrium is approached)

Now, when I am talking about the different reactions which are simultaneously going on in any living system it follow the laws of thermodynamics, now what are the law laws of thermodynamics, that energy cannot be created nor be destroyed, but it can only be transformed from one form of energy to another that means, from potential energy to the kinetic energy. Now, where we also talk about any reactions which are going on inside any living cell, we are talking about the entropy or that disorderness of the system, entropy is the extent of disorder or the randomness of the system and becomes maximum as equilibrium is approached.

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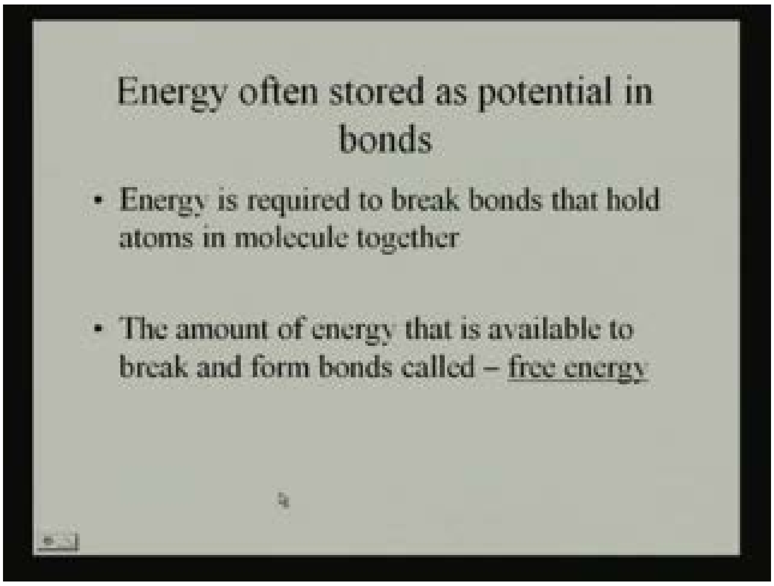

Energy often stored as potential in bonds

- Energy is required to break bonds that hold atoms in molecule together
- The amount of energy that is available to break and form bonds called – free energy

Now, this energy often stored as potential in the form of bond; now this energy is required to break the bonds and hold the atoms in the molecule together that means, is it tries to aggregate, the amount of energy that is available to break such bonds, and form such bond are called the free energy.

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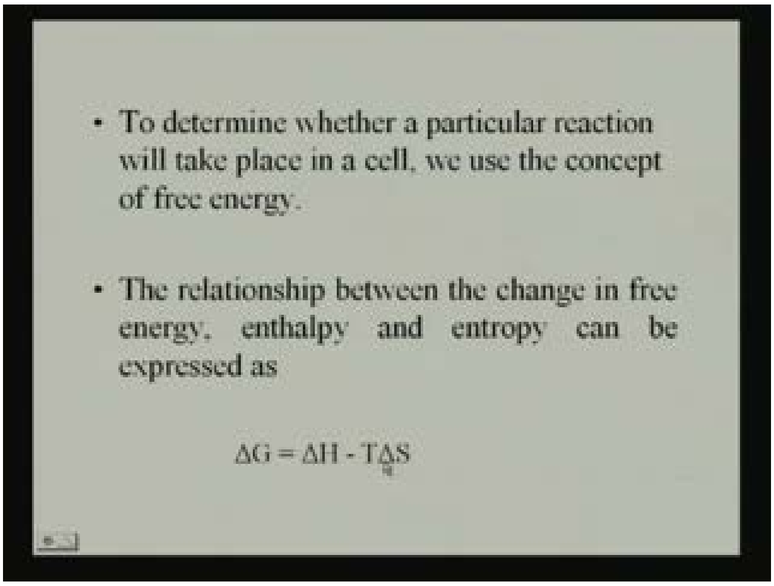

- To determine whether a particular reaction will take place in a cell, we use the concept of free energy.
- The relationship between the change in free energy, enthalpy and entropy can be expressed as

$$\Delta G = \Delta H - T\Delta S$$

Now, what is this free energy it is symbolised as delta G, now to determine whether a particular reaction will take place in a cell, we use the concept of free energy. The if you see, the relationship of this free energy enthalpy and entropy, then it can be expressed as delta G is equal to delta H minus T delta S, when delta H is the change in enthalpy, T is the temperature and delta S is the change in the entropy.

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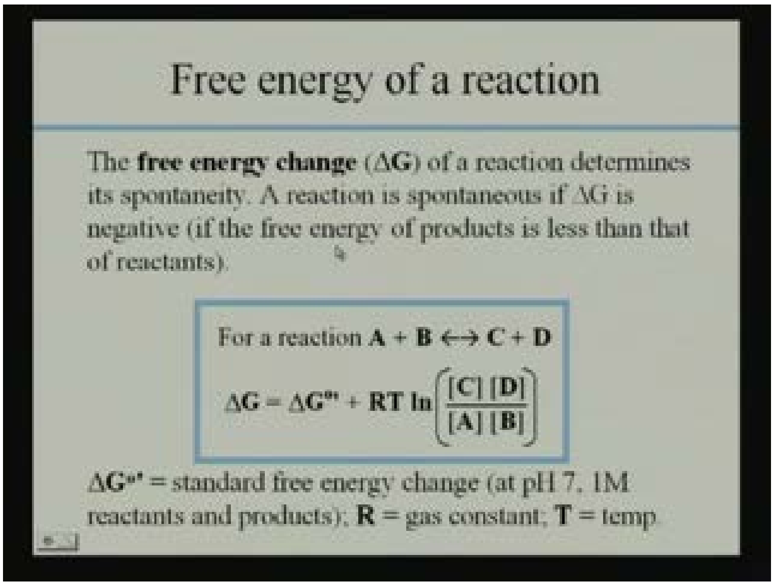

Free energy of a reaction

The **free energy change** (ΔG) of a reaction determines its spontaneity. A reaction is spontaneous if ΔG is negative (if the free energy of products is less than that of reactants).

For a reaction **A + B ⟷ C + D**

$$\Delta G = \Delta G^{0\prime} + RT \ln\left(\frac{[C]\,[D]}{[A]\,[B]}\right)$$

$\Delta G^{0\prime}$ = standard free energy change (at pH 7, 1M reactants and products); **R** = gas constant; **T** = temp

Now, when we are talking about the free energy of any reaction, now this free energy change that is the delta G of any reaction determine its spontaneous nature, spontaneity of any reaction. A reaction is spontaneous and it is favorable if the delta G value is negative that means, if the free energy of products are less than the reactant then only we can tell that this delta G is, delta G when it comes as a negative that means, it is a spontaneous reaction that means, that reaction is feasible in nature.

Now, for any reaction suppose, if we take the reactants as A plus B which is, which gives rise to C plus D which of the product, then we can find that delta G of this reaction is delta G 0 prime plus R T l n concentration of the product versus the by the concentration of reactant. When delta G 0 prime is the standard free energy change at p H 7, 1 molar reactants and products, where R is the gas constant and T is the temperature of the reaction, now when we are talking about there, if there is no mention of the temperature.

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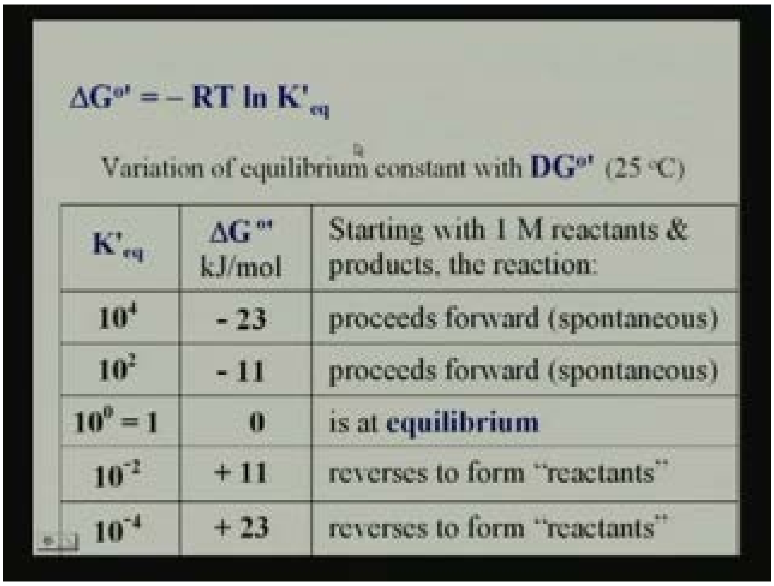

$\Delta G^{o\prime} = - RT \ln K'_{eq}$

Variation of equilibrium constant with DG°' (25 °C)

K'_{eq}	$\Delta G^{o\prime}$ kJ/mol	Starting with 1 M reactants & products, the reaction:
10^4	- 23	proceeds forward (spontaneous)
10^2	- 11	proceeds forward (spontaneous)
$10^0 = 1$	0	is at **equilibrium**
10^{-2}	+ 11	reverses to form "reactants"
10^{-4}	+ 23	reverses to form "reactants"

Now, here we can we can think of that reaction has taken place for any biological system at 37 degree celsius. Now, here delta G 0 prime is negative R T l n K equilibrium, now when we are talking about there if there is no mention of the temperature.

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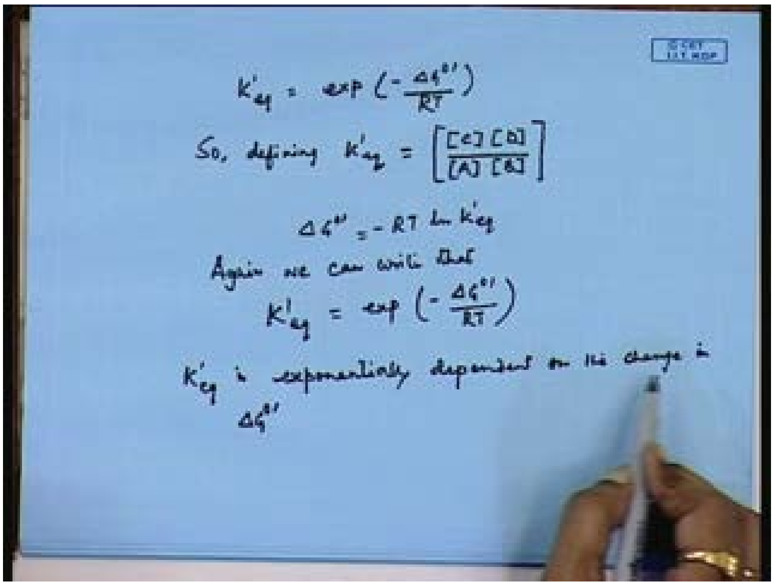

$K'_{eq} = \exp\left(-\frac{\Delta G^{0\prime}}{RT}\right)$

So, defining $K'_{eq} = \left[\frac{[C][D]}{[A][B]}\right]$

$\Delta G^{0\prime} = - RT \ln K'_{eq}$

Again we can write that

$K'_{eq} = \exp\left(-\frac{\Delta G^{0\prime}}{RT}\right)$

K'_{eq} is exponentially dependent on the change in $\Delta G^{0\prime}$

Now, how it has come as we have already, I have already shown, now You the delta G is equal to delta G is equal to delta G 0 prime plus R T l n concentration of product by concentration of reactant, now here at equilibrium, we know delta G are the free energy of any reaction is 0, now if this is so then we can write this equation as 0 is equal to delta G 0 prime plus R T l n and concentration of product by concentration of reactant.

Or it can be rewritten as delta G 0 prime is equal to minus R T l n concentration of product by concentration of reactant or K equilibrium is the concentration of product by the concentration of the reactant. So, we can write and if K prime K prime equilibrium is denoted as concentration of product by concentration of reactant, then we can rewrite the delta G 0 prime is equal to minus R T l n K equilibrium.

Now, when we are talking about this particular K equilibrium it is the exponential of delta G 0 prime by RT, so when we are defining the K equilibrium that is the concentration of product, by the concentration of reactant delta G 0 is equal to R T l n in to K equilibrium. It can be otherwise, written as that K equilibrium is the exponential of delta G 0 prime by R T that means, K equilibrium is exponentially dependent on the change of this delta G 0 prime, now when we are taking about this, the different variation of equilibrium constant at delta G 0 prime at 25 degree centigrade.

So, here the reaction which is carried out at 25 degree centigrade and if we see that K equilibrium and delta G 0 prime that is kilo joule per mole and if we see, the starting of the, the starting with 1 molar reactant and product in the reaction, then we can find that that here if if you see that, the order of reaction here this K equilibrium is 10 to power 4 and here, this delta G is only this minus 23 kilo joule per mole, now we have to have the a very serious notice of this l n, that is the exponential part.

And if the change in this, the order of reaction is in the form of 100 if order, the change in K equilibrium is nearly by the single unit say here, the order of this reaction is 100 at 100, if and here there is some minor negligible change in the delta G 0 prime, and this is when this delta G 0 prime is negative, it indicates the reaction is a spontaneous and it proceeds forward direction.

At equilibrium delta G 0 prime is 0 and when it is in the positive, delta G 0 is a positive it indicates that the reverse, it is just reverse of this earlier equation, earlier conditions of the cell and it is telling that it is the non-spontaneous reaction; now here this is the indication of this K prime equilibrium and delta G for any reaction.

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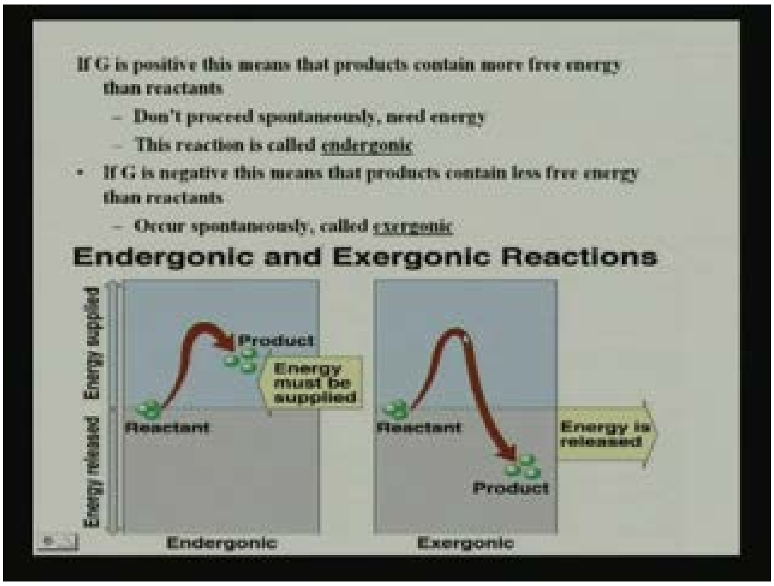

If G is positive this means that products contain more free energy than reactants

- Don't proceed spontaneously, need energy
- This reaction is called <u>endergonic</u>

- If G is negative this means that products contain less free energy than reactants
  - Occur spontaneously, called <u>exergonic</u>

Endergonic and Exergonic Reactions

Now, as I have already mention that if delta G is positive, this means that product contain more free energy than the reactant and it do not proceed spontaneously, and it needs energy, this reactions are called endergonic reaction. Now, here you see, this is the condition, if we are taking this the this particular reaction condition, here it is the reactant and reactant is converted to the product; now here to carry out this reaction that energy is supply, now energy is being utilize to break this bond to convert the reactant to the product.

Now, here if this product is formed, you see this product has got more energy than this reactant and when this product has got more energy than this reactant, then it in any point of time it may happen that this reaction may revert back or it may go for some other intermediate product formation or coupling reactions and so on; and this product is considered to be a unstable product, where as in this condition here also energy is needed to start the reaction to initiate the reaction.

And here, we can see that this particular when energy is given to this system, it is converted to this product and when this product is formed you see, the energy which was required to convert this reactant is a much much lower form then this initial reaction and that is the that is the indication that delta G of this particular condition is negative, and process is feasible. Now, here this energy is being released and here in this case, it is just reverse, here the energy is being supplied, now when we are talking about this type of reaction, we are mainly talking about the activation energy.

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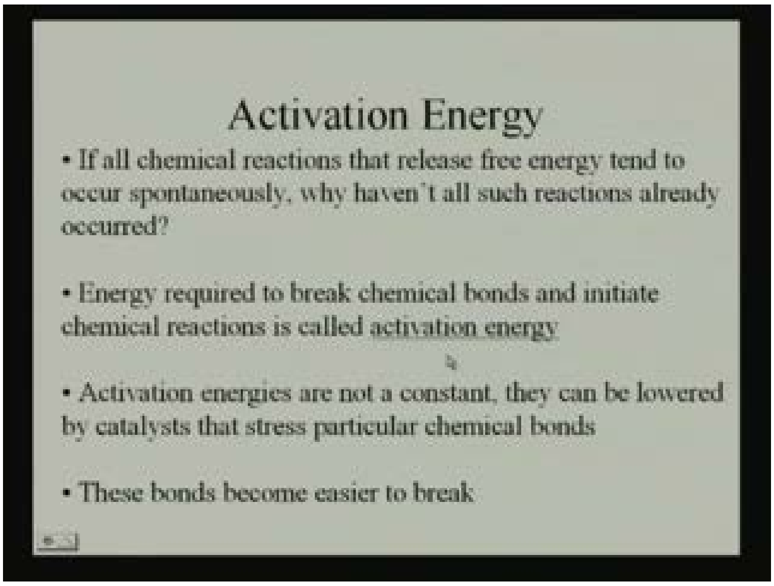

Activation Energy

- If all chemical reactions that release free energy tend to occur spontaneously, why haven't all such reactions already occurred?
- Energy required to break chemical bonds and initiate chemical reactions is called activation energy
- Activation energies are not a constant, they can be lowered by catalysts that stress particular chemical bonds
- These bonds become easier to break

Now, what is this activation energy, if all chemical reactions that release free energy tend to occurs spontaneously, why have not all such reactions already occurred, now the energy required to break the chemical bonds and to initiate the chemical reaction is called the activation energy. Now, activation energies are not a constant, but they can lower the catalysts, the catalytic requirement that stress the particular chemical bond and these bonds become easy to break.

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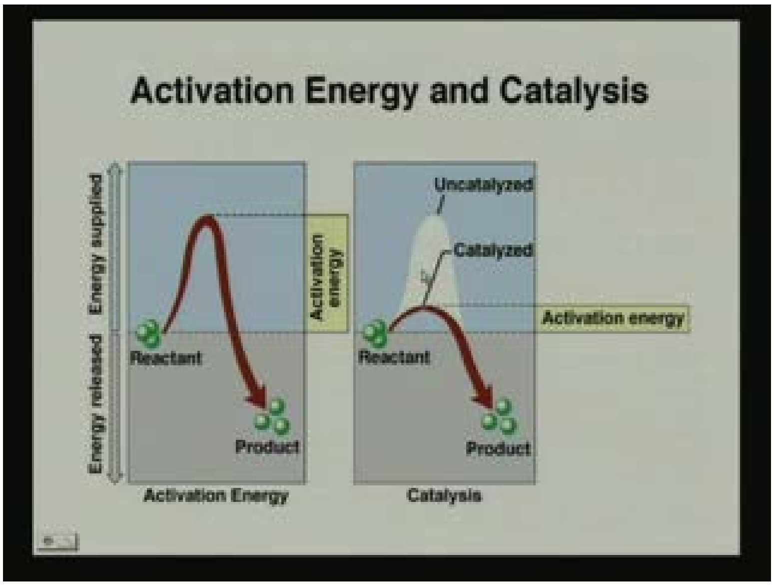

Now here, if you see two such reactions, one is catalyzed by the catalyst and one reaction reaction if we see that without the help of any catalyst. Then we can find that say reaction is taking place and when it is reaching to this peak it is the activation energy, this activation energy which is required to transform this reactant to the product which is stabilized.

Now, in another situation when in presence of catalyst the is reaction is taking place it needs much lower energy that means, activation energy to reach the much lower energy is required to reach the transition state and then the product is being produce and when such type of product formation is there, it is called in presence of the catalyst. And enzyme mediated any such reactions are very very spontaneous and transition step energy or the activation energy is a much lower compared to the uncatalyzed reaction.

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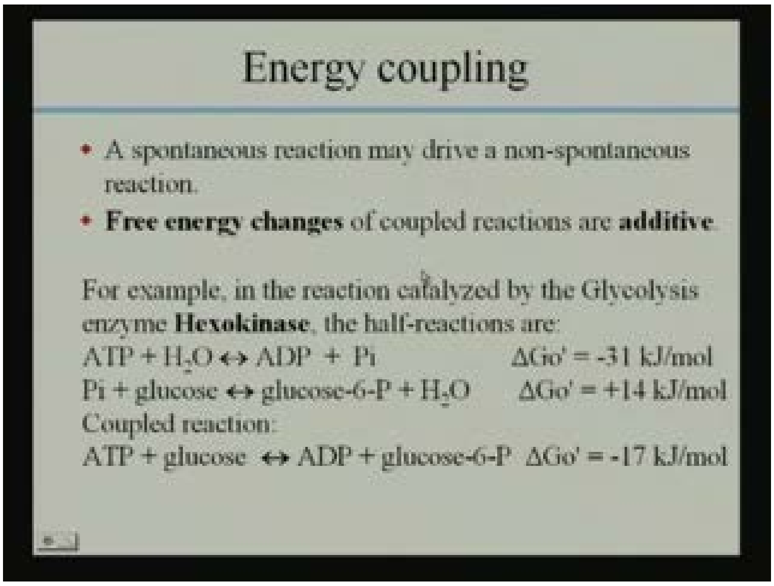

Energy coupling

- A spontaneous reaction may drive a non-spontaneous reaction.
- **Free energy changes** of coupled reactions are **additive**.

For example, in the reaction catalyzed by the Glycolysis enzyme **Hexokinase**, the half-reactions are:
$ATP + H_2O \leftrightarrow ADP + Pi \quad \Delta Go' = -31\ kJ/mol$
$Pi + glucose \leftrightarrow glucose\text{-}6\text{-}P + H_2O \quad \Delta Go' = +14\ kJ/mol$
Coupled reaction:
$ATP + glucose \leftrightarrow ADP + glucose\text{-}6\text{-}P \quad \Delta Go' = -17\ kJ/mol$

Now, here when we are taking about this energy coupling a spontaneous reaction may drive a non-spontaneous reaction. Free energy changes of coupled reactions are additives for example, when we are talking about the past stage of glycolytic process, now this particular step I will be coming little later. Now, here I want tell you Hexokinase is the enzyme which is catalysing this reaction, and in this reaction what is happening, glucose is getting phosphorylated and it is converted to glucose 6-phosphates.

Now, here if we see the reaction, the reaction is catalyzed in a glycolytic process by the enzyme hexokinase and if we see the half reactions, then we can find that ATP plus water is giving rise to ADP plus P i and if we see the delta G prime, G 0 prime it is negative and minus 31 kilo joule per mole. Now, when we are talking about the second half, then this inorganic phosphate plus glucose is getting converted to glucose 6-phosphate plus 1 molecule of water, which is the delta G is positive and 14 kilo joule per mole.

Now, if we coupled this reaction then we will find that this ATP plus glucose is getting converted to ADP plus glucose 6-phosphate, this is the ultimate product. Now, if we breakdown the half reaction then we can find that one is the spontaneous reaction another is the non-spontaneous, but when we couple this two reactions, we will find that it is the ultimate result is the spontaneity of this particular reaction.

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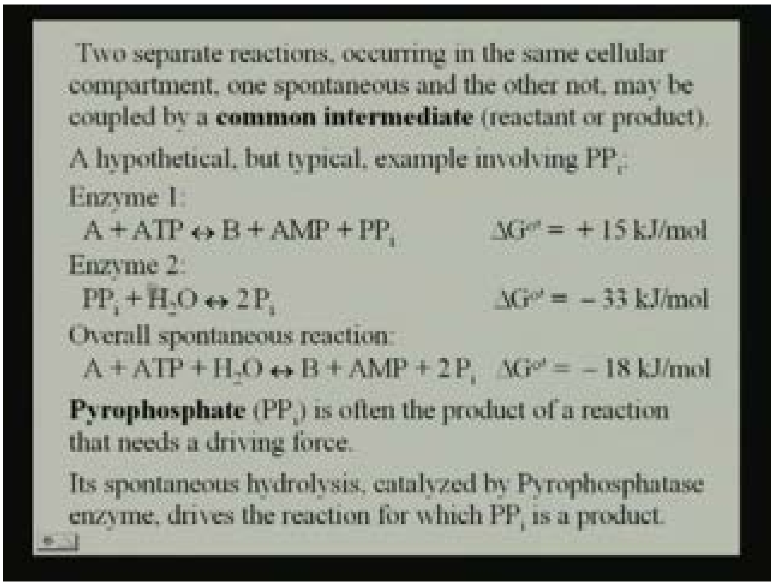

Two separate reactions, occurring in the same cellular compartment, one spontaneous and the other not, may be coupled by a **common intermediate** (reactant or product).

A hypothetical, but typical, example involving PP_i:

Enzyme 1:

$A + ATP \leftrightarrow B + AMP + PP_i$ $\Delta G^{o\prime} = +15$ kJ/mol

Enzyme 2:

$PP_i + H_2O \leftrightarrow 2P_i$ $\Delta G^{o\prime} = -33$ kJ/mol

Overall spontaneous reaction:

$A + ATP + H_2O \leftrightarrow B + AMP + 2P_i$ $\Delta G^{o\prime} = -18$ kJ/mol

Pyrophosphate (PP_i) is often the product of a reaction that needs a driving force.

Its spontaneous hydrolysis, catalyzed by Pyrophosphatase enzyme, drives the reaction for which PP_i is a product.

Now, two separate reactions occur in a same cellular form compartment, and one spontaneous and other is not, may be coupled by a common intermediate that is the reactant or the product. A hypothetical, but typical, example involve in pyrophosphate, now enzyme 1, is if we are considering enzyme 1, then it is converting converting A plus ATP which is getting convert to B plus A M P plus T P I that is pyrophosphate and delta G 0 prime is 15 kilo joule per mole; when we are talking about the second part, then we are talking about this P P i, which is the end product of the fast plus water molecule it is being convert, it to 2 P i it is the spontaneous reaction.

Now, when we are summing up this two reaction, now overall spontaneous reaction if we sum up, then we can write a plus ATP plus water is resulting B plus AMP plus 2 P i and it is a spontaneous reaction. Now, pyrophosphate is often the product of a reaction that needs a driving force, its spontaneous hydrolysis catalyzed by the pyrophosphates enzyme, drives the reaction for each the pyrophosphate is a product.

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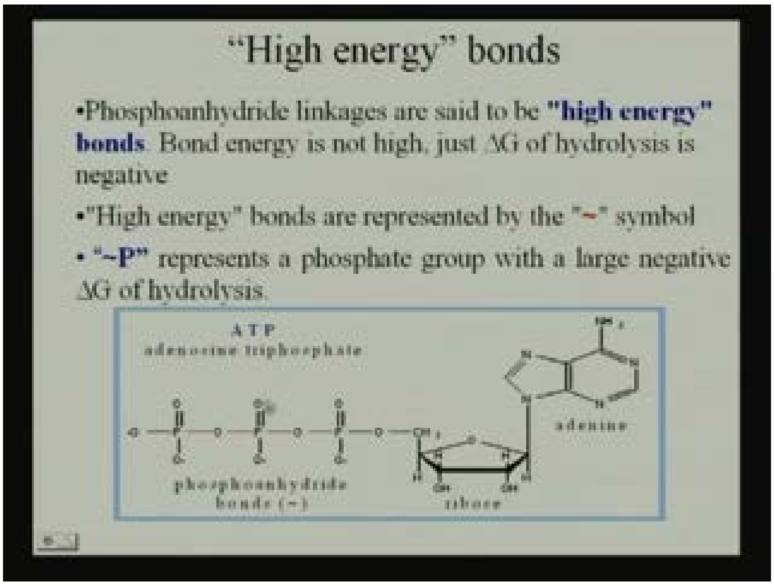

“High energy” bonds

- Phosphoanhydride linkages are said to be **"high energy" bonds**. Bond energy is not high, just ΔG of hydrolysis is negative
- "High energy" bonds are represented by the "~" symbol
- **"~P"** represents a phosphate group with a large negative ΔG of hydrolysis.

Now obviously, we are talking about high energy bond, now phosphoanhydride linkages linkages are the are said to be the high energy bond which is present in the ATP molecule, now here bond energy is not high just delta G of hydrolysis is negative. Now, when we are talking about the high energy bonds, which is a represented by this particular symbol and its represent the high energy phosphate group with a large negative change in the delta G of hydrolysis.

Now, here it is the adenine moieties, which is attach to this ribose sugar that first carbon of this ribose sugar is occupied by the adenine molecule, and to the fifth carbon of this ribose sugar, three phosphate groups are attached; and they are attached with the phosphoanhydride linkages. And this is the ATP molecule, which is there in the cell and we are we generally referred that, this form of the energy, which is being utilized by the living system.

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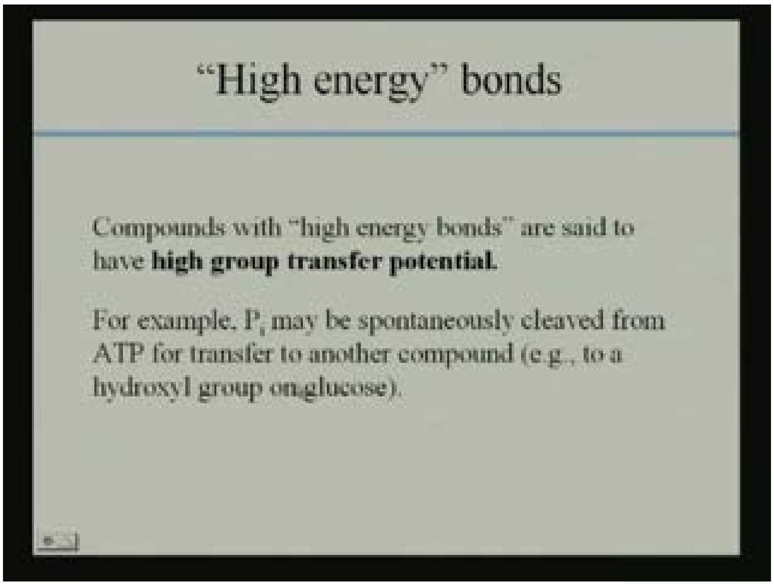

“High energy” bonds

Compounds with “high energy bonds” are said to have **high group transfer potential.**

For example, P_i may be spontaneously cleaved from ATP for transfer to another compound (e.g., to a hydroxyl group on glucose).

The compound with high energy bond are said to have high group of transfer potential for example, inorganic phosphate may be spontaneously cleaved from ATP for transfer to another compound and hydroxyl, to a hydroxyl group on glucose.

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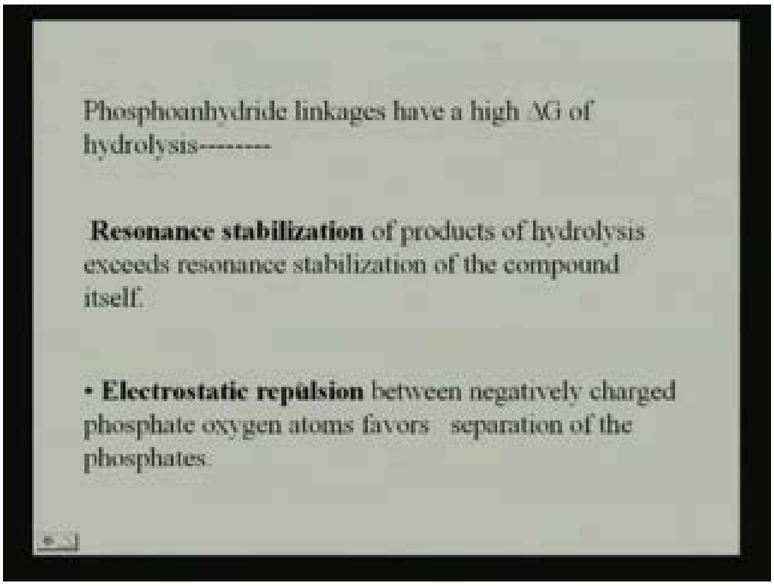

Phosphoanhydride linkages have a high ΔG of hydrolysis--------

Resonance stabilization of products of hydrolysis exceeds resonance stabilization of the compound itself.

- **Electrostatic repulsion** between negatively charged phosphate oxygen atoms favors separation of the phosphates.

Now, if we see the phosphoanhydride linkages which have a very high delta G of hydrolysis, then we can find that, because of this resonance stabilization of the product of hydrolysis which exceed the resonance stabilization of the compound itself. And also we can find that, the electrostatic repulsion between the negatively charged phosphate oxygen atoms favors the separation of the phosphate, and that is the reason of the phosphoanhydride linkages which have the very high delta G valid.

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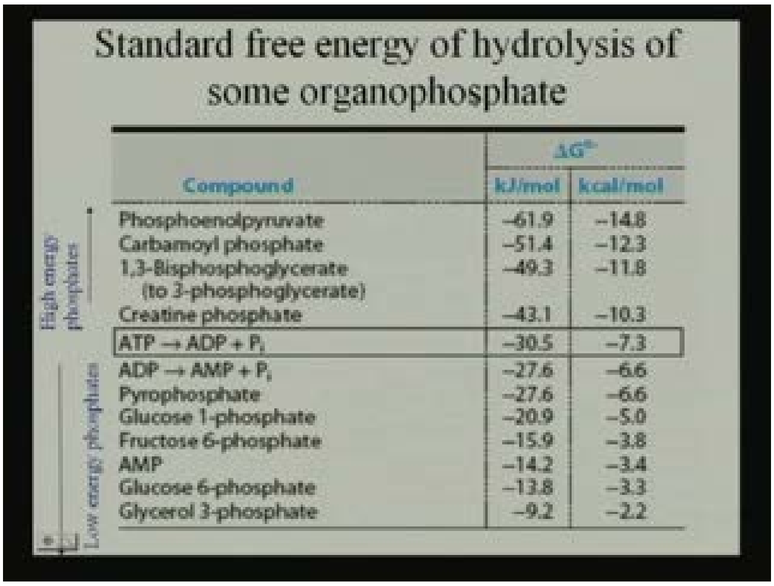

Standard free energy of hydrolysis of some organophosphate

Compound	$\Delta G'^{\circ}$ kJ/mol	$\Delta G'^{\circ}$ kcal/mol
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP → ADP + P_i	−30.5	−7.3
ADP → AMP + P_i	−27.6	−6.6
Pyrophosphate	−27.6	−6.6
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
AMP	−14.2	−3.4
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-phosphate	−9.2	−2.2

Now, if we take this ATP as the standard then, we can find that it has got low energy phosphates as well as high energy phosphate, now when we are talking about the phosphoenolpyruvate, carbamoyl, phosphate 1, 3-bisphosphoglycerate to 3-phosphoglycerate, creatine phosphate etcetera. These reactions will find that, this particular energies, which are taking which are produced during during the reactions are coming under the high phosphate bond, high phosphate energy.

And this these particular reactions are otherwise known as substrate level phosphonalysation which are these coming later, and here the some of this reactions of which are their taking place within a system are the low energy phosphate. So, these are the general categorisation of the standard free energy of hydrolysis of some organophosphate compound, based on this delta G value, we can differentiate this type of, we can categories in this fashion.

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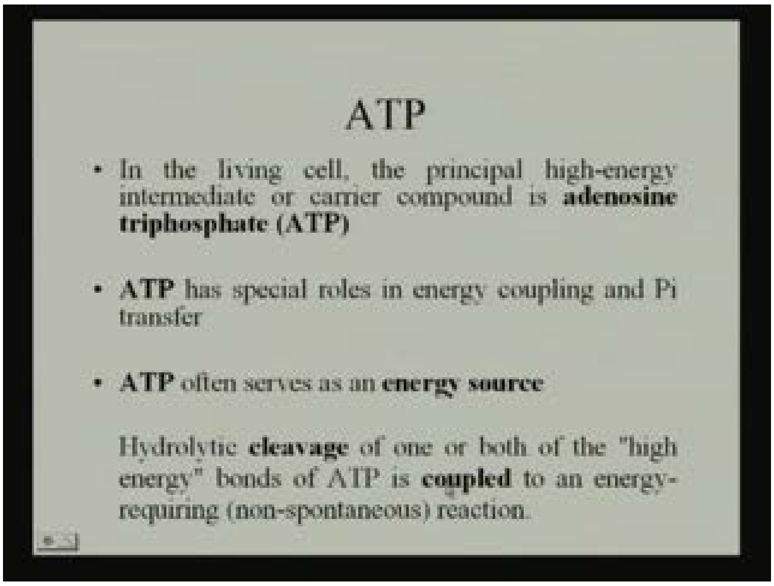

ATP

- In the living cell, the principal high-energy intermediate or carrier compound is **adenosine triphosphate (ATP)**
- **ATP** has special roles in energy coupling and Pi transfer
- **ATP** often serves as an **energy source**

  Hydrolytic **cleavage** of one or both of the "high energy" bonds of ATP is **coupled** to an energy-requiring (non-spontaneous) reaction.

Now, when we are talking about this ATP in the living cell the principle energy high-energy intermediate or the carrier compound is adenine triphosphate, adenine triphosphate has a special role in energy coupling and the inorganic phosphate transfer. ATP often serves as the energy source and hydrolytic cleavage of one or both the high energy bonds of ATP is coupled to an energy requiring non-spontaneous reaction that means, we can expect two phosphate group which may be cleave by the ATP molecule.

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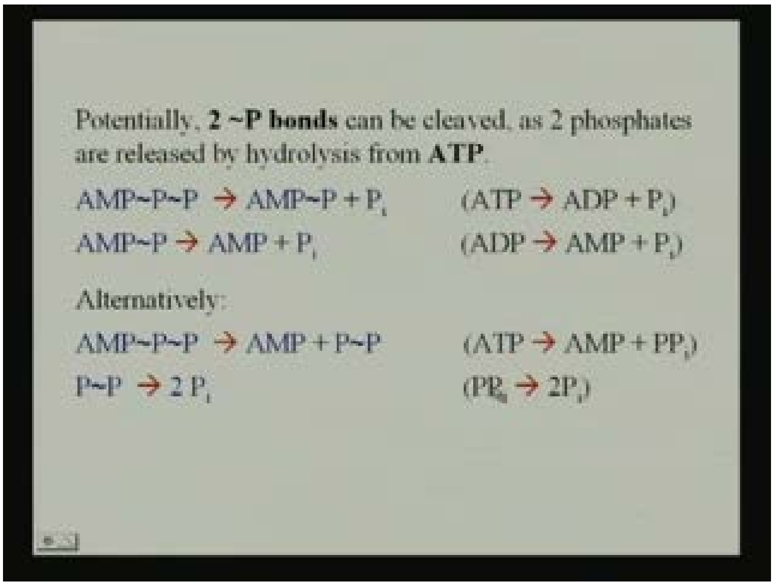

Potentially, **2 ~P bonds** can be cleaved, as 2 phosphates are released by hydrolysis from **ATP**.

$\mathrm{AMP{\sim}P{\sim}P} \rightarrow \mathrm{AMP{\sim}P} + P_i$ $(\mathrm{ATP} \rightarrow \mathrm{ADP} + P_i)$

$\mathrm{AMP{\sim}P} \rightarrow \mathrm{AMP} + P_i$ $(\mathrm{ADP} \rightarrow \mathrm{AMP} + P_i)$

Alternatively:

$\mathrm{AMP{\sim}P{\sim}P} \rightarrow \mathrm{AMP} + \mathrm{P{\sim}P}$ $(\mathrm{ATP} \rightarrow \mathrm{AMP} + PP_i)$

$\mathrm{P{\sim}P} \rightarrow 2\,P_i$ $(PP_i \rightarrow 2P_i)$

Now, if we see some reaction that 2-phosphate group, can be cleaved as 2-phosphates are released by the hydrolysis of ATP as I have told, that AMP which is having 2 phosphate that means, in other way we can tell ATP and when ATP is getting d phosphorylation reactions are going on we can get AMP or having P or we can delete that adenine diphosphate and 1 P i and when ADP is AMP I linked P is further depleted it forms AMP plus P i that means, ADP to AMP and P i. Alternatively AMP and 2-phosphate is gives gives rise to AMP plus 2-phosphate together which is further convert it to 2 inorganic phosphate that means, this pyrophosphate is convert it to inorganic phosphate and TPI is giving rise to two P i.

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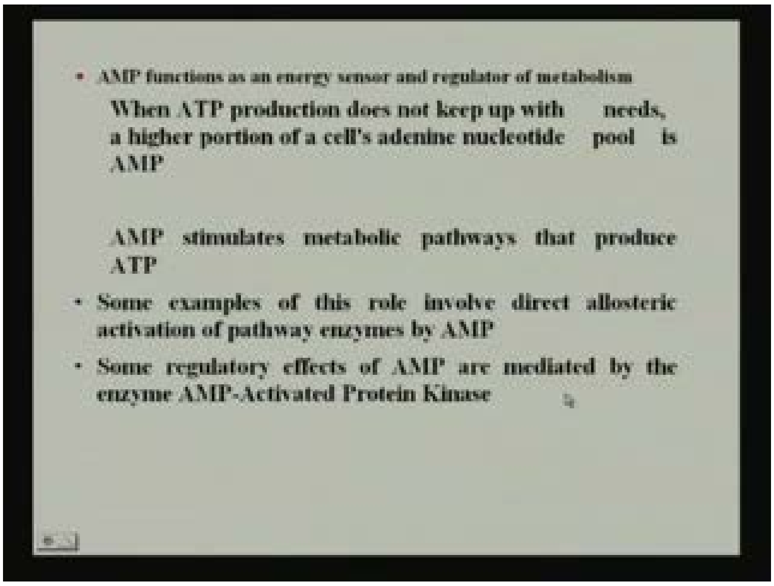

- AMP functions as an energy sensor and regulator of metabolism

  When ATP production does not keep up with needs, a higher portion of a cell's adenine nucleotide pool is AMP

  AMP stimulates metabolic pathways that produce ATP
- Some examples of this role involve direct allosteric activation of pathway enzymes by AMP
- Some regulatory effects of AMP are mediated by the enzyme AMP-Activated Protein Kinase

Now, AMP functions as an energy sensor and regulator of any metabolic activities, when ATP production does not keep up with the need a higher portion of the cells adenine nucleotide pool is the AMP. AMP stimulate the metabolic pathway that produce ATP and some example of this role, which involve direct allosteric activation of the pathway enzyme by the AMP, some regulatory effects of AMP are mediated by the enzyme that is AMP activated protein kinase.

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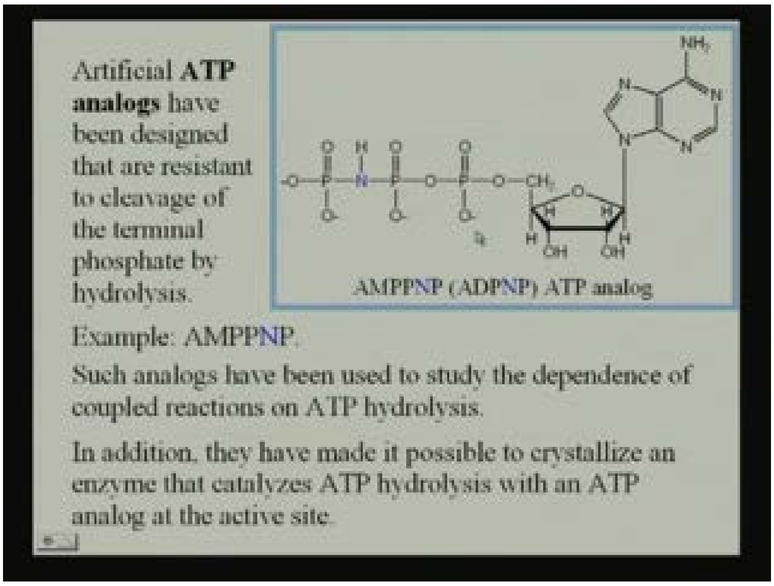

Artificial **ATP analogs** have been designed that are resistant to cleavage of the terminal phosphate by hydrolysis.

AMPPNP (ADPNP) ATP analog

Example: AMPPNP.

Such analogs have been used to study the dependence of coupled reactions on ATP hydrolysis.

In addition, they have made it possible to crystallize an enzyme that catalyzes ATP hydrolysis with an ATP analog at the active site.

Now, here as I have already told you about the ATP, some artificial ATP analogs have been designed that are have resistant to cleave the terminal phosphate right hydrolysis. Now, what is that molecule, it is called AMPPNP such analogy have been used to study the dependence of coupled reaction on ATP hydrolysis, in addition they have made it possible to crystallize and enzyme that catalyzes ATP hydrolysis with and ATP analog at the active site. So, you can see here, one nitro group is being attached to these particular moieties to resist the cleavage of the terminal phosphate group of ATP and this is called the ATP analog.

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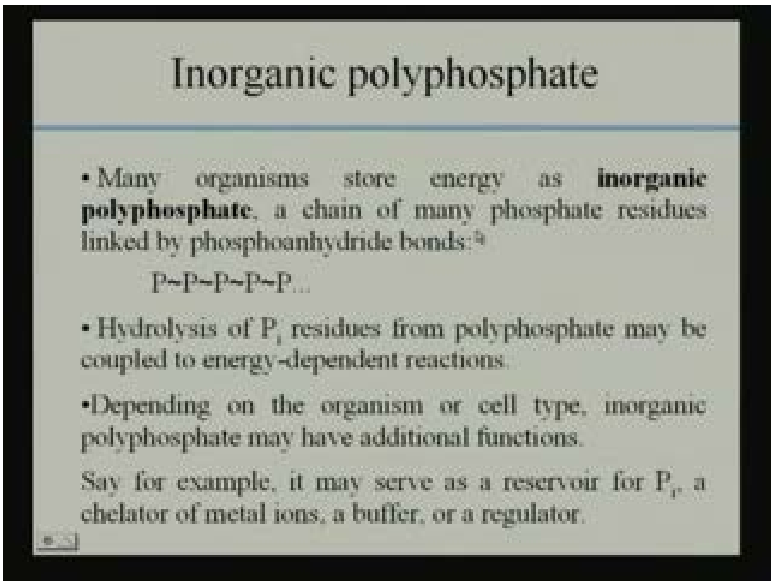

Inorganic polyphosphate

- Many organisms store energy as **inorganic polyphosphate**, a chain of many phosphate residues linked by phosphoanhydride bonds:

  P~P~P~P~P…

- Hydrolysis of P_i residues from polyphosphate may be coupled to energy-dependent reactions.
- Depending on the organism or cell type, inorganic polyphosphate may have additional functions.

Say for example, it may serve as a reservoir for P_i, a chelator of metal ions, a buffer, or a regulator.

Now, inorganic phosphate in many substances, in many organisms they store the energy as inorganic polyphosphate, a chain of many phosphate residues are linked together like P P P P and in these way and it it forms the phosphoanhydride bond. Hydrolysis of P i residues from polyphosphate may be coupled to energy dependent reaction, depending on the organism or cell type inorganic polyphosphate may have the additional functions. Say for example, it may serve as the reservoir of P i, a chelator of metal ions a buffer or a regulator of any reaction.

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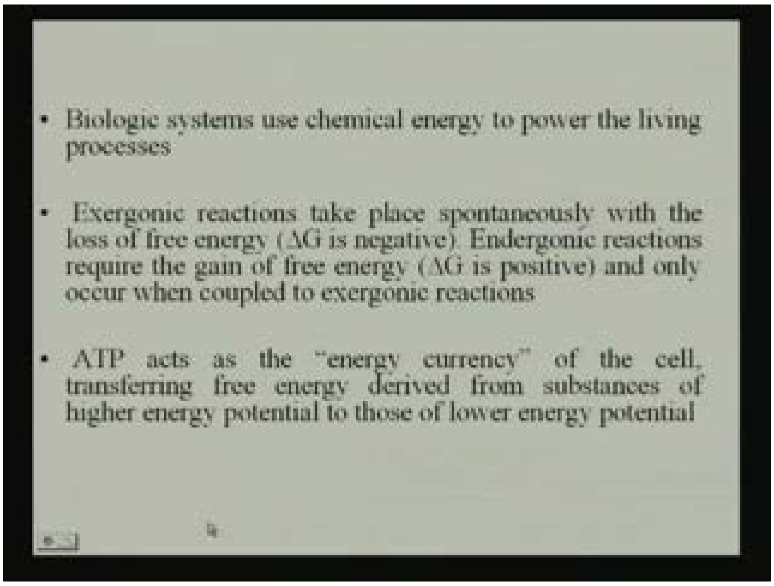

- Biologic systems use chemical energy to power the living processes
- Exergonic reactions take place spontaneously with the loss of free energy (ΔG is negative). Endergonic reactions require the gain of free energy (ΔG is positive) and only occur when coupled to exergonic reactions
- ATP acts as the "energy currency" of the cell, transferring free energy derived from substances of higher energy potential to those of lower energy potential

So, if we see the biological system the living system, use the chemical energy to power the living the living process, exergonic reaction takes place simultaneously with the loss of free energy that means, the delta G is negative. And endergonic reaction requires to gain the free energy that is delta G is positive and only occur when coupled to exergonic reaction, ATP acts as the energy currency of the cell transferring the free energy derived from substances of higher energy potential to those of lower energy potential.

And this way the different reactions, that different catalytic reaction are studies out in any living system, now here with this I am just concluding this bioenergetics. Now, will be coming to the actual reaction sustain in the living cell which is being carried out in some of the metabolic activities.

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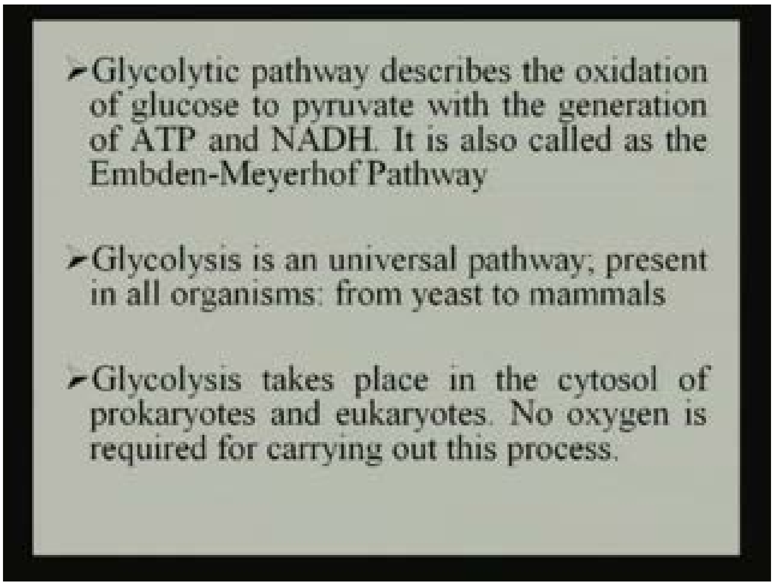

- Glycolytic pathway describes the oxidation of glucose to pyruvate with the generation of ATP and NADH. It is also called as the Embden-Meyerhof Pathway
- Glycolysis is an universal pathway; present in all organisms: from yeast to mammals
- Glycolysis takes place in the cytosol of prokaryotes and eukaryotes. No oxygen is required for carrying out this process.

Glycolysis is one of such example, where glycolytic pathway describes the oxidation of glucose to pyruvate, pyruvic acid with the generation of ATP and NADH, and it is also otherwise known as Embden-Meyerhof pathway. Glycolysis is a the universal pathway, it is present in all living organisms it from, starting from yeast to mammals, this is the process which takes place in the cytosol, cytoplasmic (()) of the cell, of the prokaryotes and eukaryotes here, no energy is required for, no oxygen is required for carrying out this particular process.

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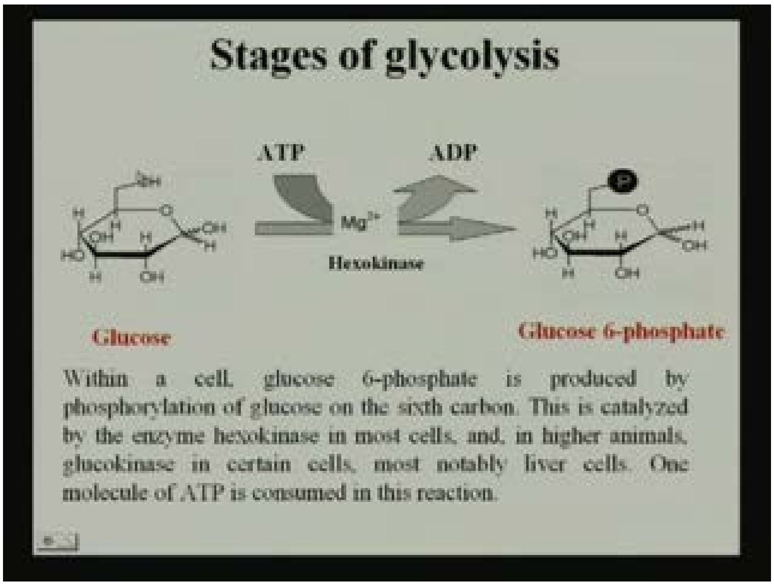

Stages of glycolysis

Within a cell, glucose 6-phosphate is produced by phosphorylation of glucose on the sixth carbon. This is catalyzed by the enzyme hexokinase in most cells, and, in higher animals, glucokinase in certain cells, most notably liver cells. One molecule of ATP is consumed in this reaction.

Now, if we see the glycolytic process the first step of glycolysis is glucose to glucose 6-phosphates, now if we see this entire reaction, the entire glycolytic process can be divided into ten different steps, now this ten different steps can be once again divided in to two states, now first stage and second stage.

So, let us start with the first stage of glycolysis, now if the first stage glucose is getting converted to glucose 6-phosphate, now within a cell glucose 6-phosphate is produced by the first phosphorylation of glucose that means, this is the glucose moieties and here glucose 6-phosphate means on the sixth carbon atom here this phosphorylation is taking place.

And here, when we are talking about this particular reaction here 1 molecule of ATP is being utilized to produce this ADP, and this phosphate which is being transferred is coming and phosphorylating this glucose molecule, which is the starting substrate for this reaction. Now, here besides this glucose there may be a some other hexose sugar which can also participate in this reaction, this reaction is catalyze by the enzyme which is called hexokinase; now in this bioenergetics I told you that, hexokinase is one of the example of glycolytic process, now here, any type of ketohexose or aldohexose can be converted to glucose 6-phosphate.

Now, here if we see the affinity of this hexokinase enzyme then we can find that, the affinity of hexokinase is more towards the aldose sugar then the ketose sugar, like mannose, galactose and so on. So, glucose and if they are converting this enzyme is get this helping to convert this, it is catalysing the reaction to convert glucose to glucose 6-phosphate; this is a this is catalysed by the enzyme hexokinase in most cell that hexokinase is present, in every cells starting from the higher animals to the lower.

Similarly, the same reaction can also be carried out with the another enzyme which is called the glucokinase, the name itself indicates that glucokinase has got more affinity then the hexokinase enzyme, this glucokinase is present in the liver cell of any liver cell of any animal and 1 molecule of ATP is being used in this particular reaction. If we compared the kinetics property of this hexokinase and this glucokinase enzyme then we can find that as glucokinase has got got only specificity towards glucose, the K m value of glucose is much much lower compared to this hexokinase enzyme.

Now, this if hexokinase enzyme is is present in the liver and it is absent in the muscle, now when glucose concentration is high glucokinase is active, but this glucokinase enzyme is absent in any diabetic patient, now this is the first stage and this both the enzyme hexokinase and glucokinase needs the metal ion like M g or (()) catalyzing this reaction.

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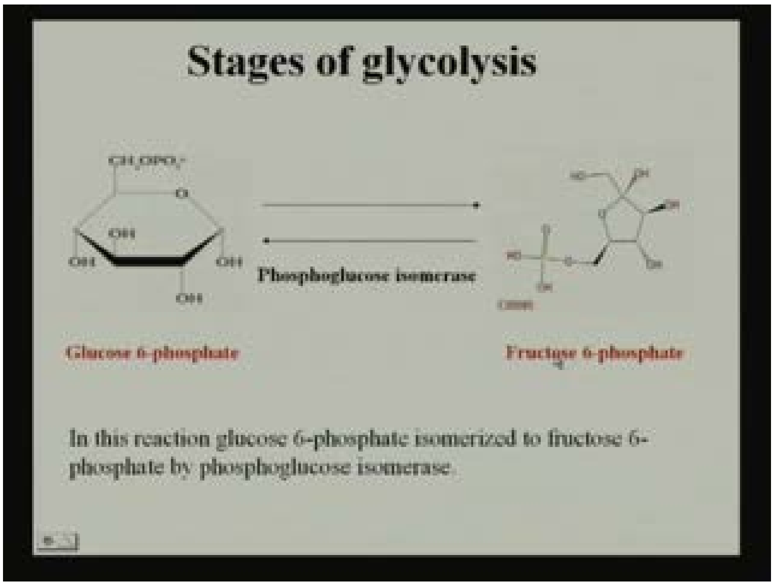

Stages of glycolysis

Phosphoglucose isomerase

Glucose 6-phosphate

Fructose 6-phosphate

In this reaction glucose 6-phosphate isomerized to fructose 6-phosphate by phosphoglucose isomerase

The second step of this glycolytic process is glucose 6-phosphate to fructose 6-phosphate, this is the isomerisation reaction phosphoglucose isomerase is the enzyme and they are converting this aldose sugar to the ketose sugar; in this reaction glucose 6-phosphate isomerase to fructose 6-phosphate by phosphoglucose isomerase.

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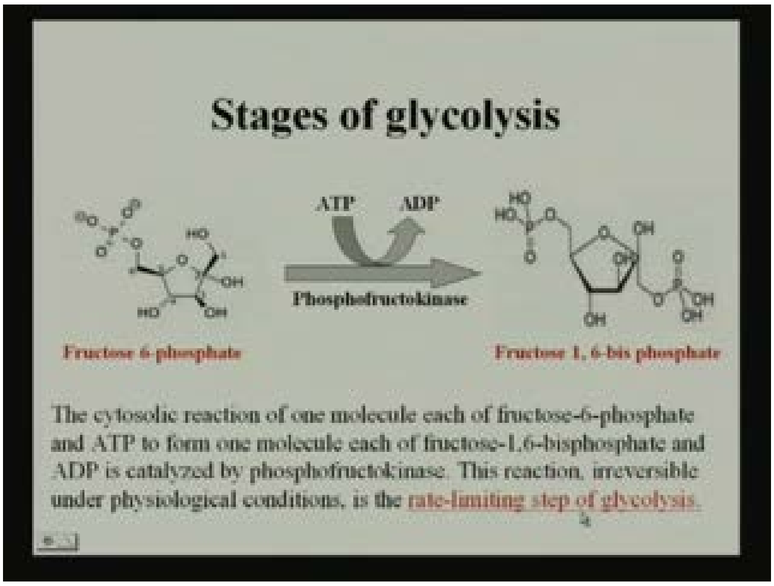

Stages of glycolysis

ATP ADP

Phosphofructokinase

Fructose 6-phosphate

Fructose 1, 6-bis phosphate

The cytosolic reaction of one molecule each of fructose-6-phosphate and ATP to form one molecule each of fructose-1,6-bisphosphate and ADP is catalyzed by phosphofructokinase. This reaction, irreversible under physiological conditions, is the rate-limiting step of glycolysis.

Now, in the third step, fructose 6-phosphate is getting converted to fructose 1, 6-bis phosphate, now in this particular reaction phosphofructokinase is the enzyme, which is playing a significant role this enzyme is so important that it is otherwise known as, the rate limiting step of glycolysis. Now, in the cytoplasmic reaction 1 molecule of molecule each of fructose 6-phosphate and 1 molecule of ATP is is converting the fructose 6-phosphate to fructose 1, 6-bis phosphate; now 1 molecule of ADP is produced in this reaction, this reaction is irreversible under physiological condition.

Now, what is happing in the first step, when glucose is phosphorylated to glucose 6-phosphate 1 molecule of ATP was utilize and see here, another molecule of ATP is being utilized for getting this fructose 6-phosphate to fructose, 1, 6-bis phosphate that means, the first carbon and the sixth carbon are getting phosphorylated with this ATP, and ATP is converted to ADP.

Now, if we see the enzyme phosphofructokinase this particular enzyme it is the allosteric enzyme, it is being huge in structure 3,80,000 is the molecular weight of this particular enzyme, it can stimulate and modulate the allosteric function and when in the cell ATP concentration along with the site rate or long chain fatty acid concentration is more it inhibits the phosphofructokinase.

And when it this enzyme is getting inhibited, the entire reaction is getting stop, when in the cell the ATP concentration is much lower and then this phosphofructokinase is once again getting stimulated or it is switched on and it is converting this fructose 6-phosphate to fructose 1, 6-bis phosphate.

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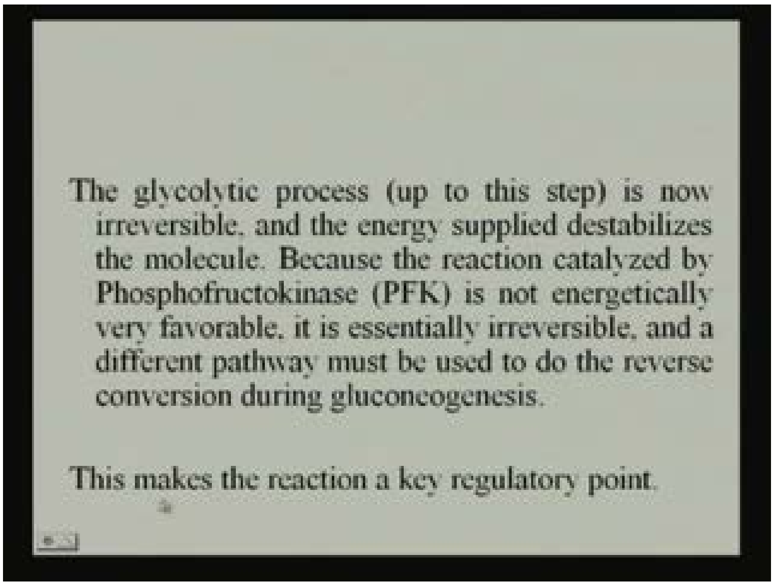

The glycolytic process (up to this step) is now irreversible. and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase (PFK) is not energetically very favorable. it is essentially irreversible. and a different pathway must be used to do the reverse conversion during gluconeogenesis.

This makes the reaction a key regulatory point.

The glycolytic process up to this step is now irreversible, and the energy supplied this stabilizes the molecule, because the reaction catalyzed by the phosphofructokinase is not energetically very favourable, it is essentially irreversible, and the different pathways must be used to use to do the reverse the conversion during gluconeogenesis, and this makes the reaction a key regulatory point of glycolytic state.

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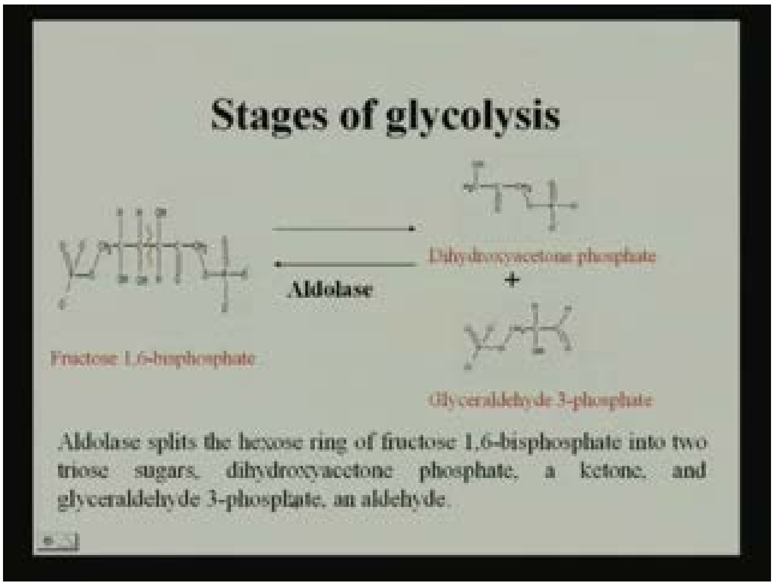

Stages of glycolysis

Aldolase splits the hexose ring of fructose 1,6-bisphosphate into two triose sugars. dihydroxyacetone phosphate. a ketone. and glyceraldehyde 3-phosplate. an aldehyde.

Now, when fructose 1, 6-bis phosphate is produced with the help of this enzyme aldolase it is getting, it is being cleaved to 2 molecule of 3 carbon compound, one is called dihydroxyacetone phosphate, another is the glyceraldehyde 3-phosphate. Now, earlier what we have seen 1, 6 carbon hexose molecule was there, now this 1 hexose molecule is now spitted to 2 molecules 3 carbon, each one is the dihydroxyacetone phosphate another is the glyceraldehyde 3-phosphate split the hexose a ring of 1, 6-bisphosphate into two triose sugar, that is the dihydroxyacetone phosphate and glyceraldehyde that is the aldose sugar.

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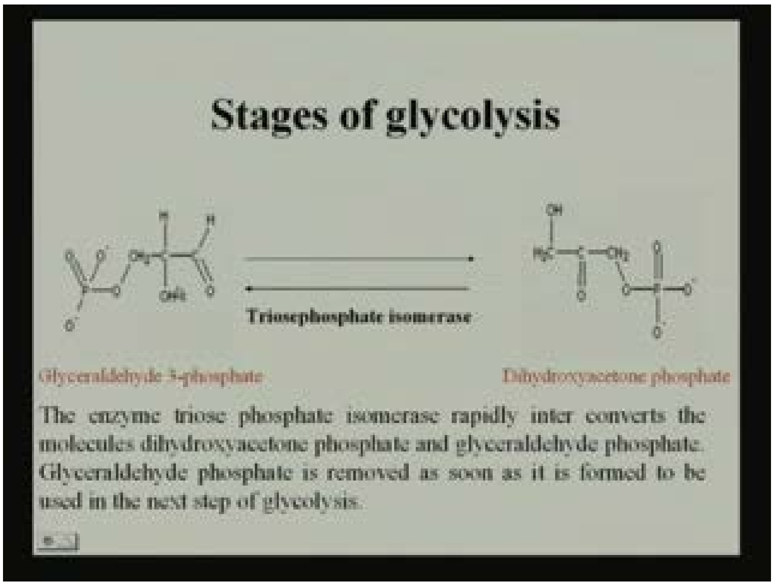

Stages of glycolysis

Triosephosphate isomerase

Glyceraldehyde 3-phosphate

Dihydroxyacetone phosphate

The enzyme triose phosphate isomerase rapidly inter converts the molecules dihydroxyacetone phosphate and glyceraldehyde phosphate. Glyceraldehyde phosphate is removed as soon as it is formed to be used in the next step of glycolysis.

Now, glyceraldehyde 3-phosphate is in the presence of triosephosphate isomerase enzyme can be once again getting converted to each other that means, isomerisation of ketose to aldose sugar is taking place very first in within the cell. The enzyme triosephosphate isomerase rapidly interconvert the molecules of dihydroxyacetone phosphate and glyceraldehyde phosphate, glyceraldehyde phosphate is removed as soon as it is formed to be used in the next step of glycolysis that means, as soon as dihydroxyacetone phosphate this ketose sugar converted to aldose sugar, this glyceraldehyde 3-phosphate is immediately being take up in the next stage of glycolytic process.

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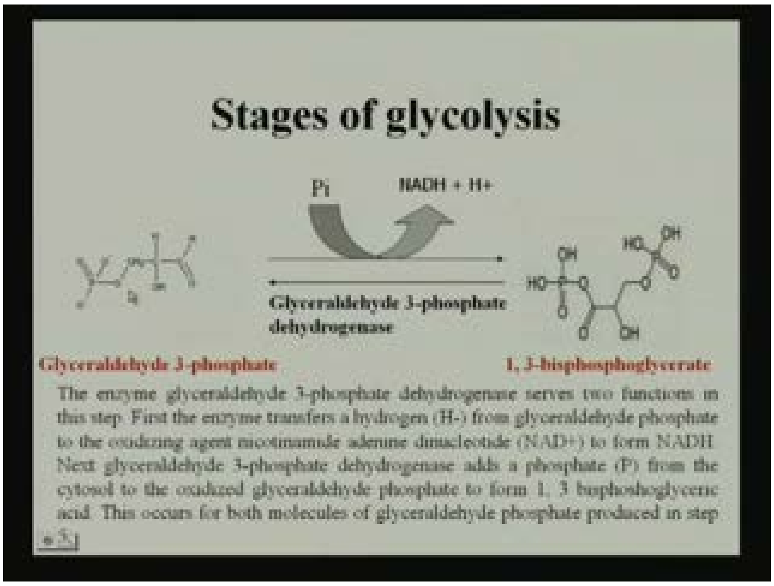

Stages of glycolysis

Pi

NADH + H+

Glyceraldehyde 3-phosphate dehydrogenase

Glyceraldehyde 3-phosphate

1, 3-bisphosphoglycerate

The enzyme glyceraldehyde 3-phosphate dehydrogenase serves two functions in this step. First the enzyme transfers a hydrogen (H-) from glyceraldehyde phosphate to the oxidizing agent nicotinamide adenine dinucleotide (NAD+) to form NADH. Next glyceraldehyde 3-phosphate dehydrogenase adds a phosphate (P) from the cytosol to the oxidized glyceraldehyde phosphate to form 1, 3 bisphoshoglyceric acid. This occurs for both molecules of glyceraldehyde phosphate produced in step

Now, if we see this glyceraldehyde 3-phosphate, this glyceraldehyde 3-phosphate in the presence of dehydrogenase enzyme it is once again getting phosphorylated to 1, 3-bisphosphoglycerate, now the enzyme glyceraldehyde 3-phosphate dehydrogenate serves two function in this particular step. The first of the enzyme transfer the hydrogen from the glyceraldehyde phosphate to the oxidizing agent of nicotinamide adenine dinucleotide that means, here this NAD is being converted to NADH.

Next glyceraldehydes 3-phosphate dehydrogenase at a phosphate from the cytosol to the oxidized glyceraldehyde phosphate to form 1, 3-bisphosphoglyceric acid, this occur for both the molecules, from what is happing, earlier we were talking about 1 hexose, now we talking about two triose sugar that means, this triose sugars are 2 molecules that is 1 hexose is converted to 2 molecules.

Now, when 1, 3-bisphosphoglycerate is produced that means, 2 molecule of such compounds where produced, so this occur for both molecules of glyceraldehyde phosphate to produced in a next step. Now, hence for whatever may be the steps, the steps where 2 molecule of each tricarbon molecules, so 1, 3-bisphosphate bisphosphoglycerate is converted to 3-phosphoglycerate with a presence of the enzyme phosphoglycerate kinase.

The enzyme phosphoglycerate kinase transfer the phosphate from 1, 3-bisphosphoglyceric acid to a molecule of ADP to form the ATP and which is called as substrate level phosphorylation that means, it is losing one phosphate from this core moieties, and ADP is being taken up and it is produced the ATP molecule, and as 2 molecule of this this 1, 3-bisphosphoglycerate is there, so 2 molecule of ATP is being produced in this particular step.

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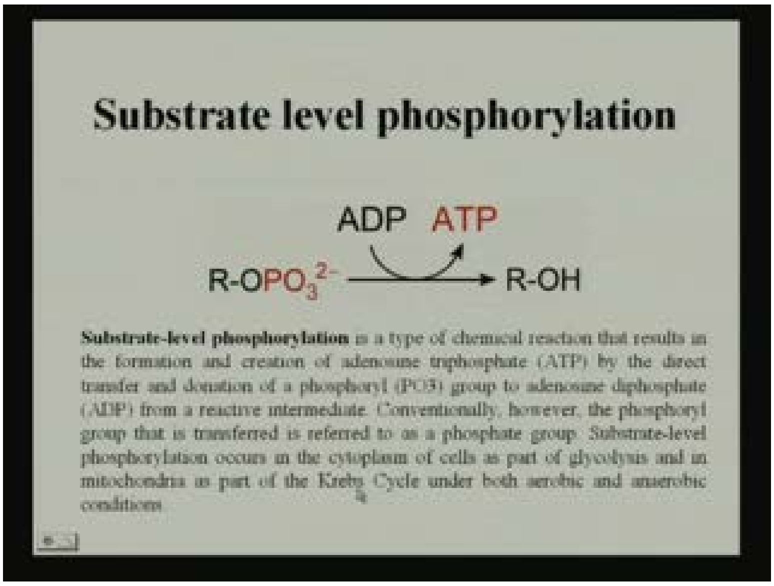

Substrate level phosphorylation

$$\text{R-OPO}_3^{2-} \xrightarrow{\text{ADP} \quad \text{ATP}} \text{R-OH}$$

Substrate-level phosphorylation is a type of chemical reaction that results in the formation and creation of adenosine triphosphate (ATP) by the direct transfer and donation of a phosphoryl (PO3) group to adenosine diphosphate (ADP) from a reactive intermediate. Conventionally, however, the phosphoryl group that is transferred is referred to as a phosphate group. Substrate-level phosphorylation occurs in the cytoplasm of cells as part of glycolysis and in mitochondria as part of the Krebs Cycle under both aerobic and anaerobic conditions.

This particular step is otherwise known as, the substrate level phosphorylation, the substrate level phosphorylation is the type of chemical reaction, that is that results in the formation of creation of ATP by the direct transfer and donation of a phosphorylation phosphate to adenine diphosphate from the reaction intermediate. And conventionally, however, the phosphorylation group that is transferred is refer to as phosphate group and for substrate-level phosphorylation occurs in the cytoplasm of the cell, as the part of that glycolytic process and the mitochondrial, in mitochondrial for the kerb cycle under the aerobic and anaerobic process.

Now, when we are talking about this 3-bisphosphoglycerate it is once again the transfer of phosphate group is taking place in the presence of the enzyme mutase and it it is converted to 2-phosphoglycerates, so second this third, from the third carbon it is transfer to the second carbon. Now, when this two phosphoglycerate is produced with the help of this mutase enzyme, the next step with help of enolase which is a releasing 1 molecule of water from this reaction, and is converting to phosphoenolpyruvate; now this phosphoenolpyruvate happens for each molecule of 2-phosphoglyceric acid.

Now, this phosphoenolpyruvate, when it converts convert it to pyruvate in the presence of pyruvate kinase, here also the phosphate group is being utilized by this ADP and 2 molecule of ATP is being being produced and it is other is another example of the substrate level phosphorylation. And this is one of the examples of the substrate level phosphorylation in the glycolytic pathway, now pyruvate as soon as it is produced pyruvate is considered to be the last end product of this particular cycle.

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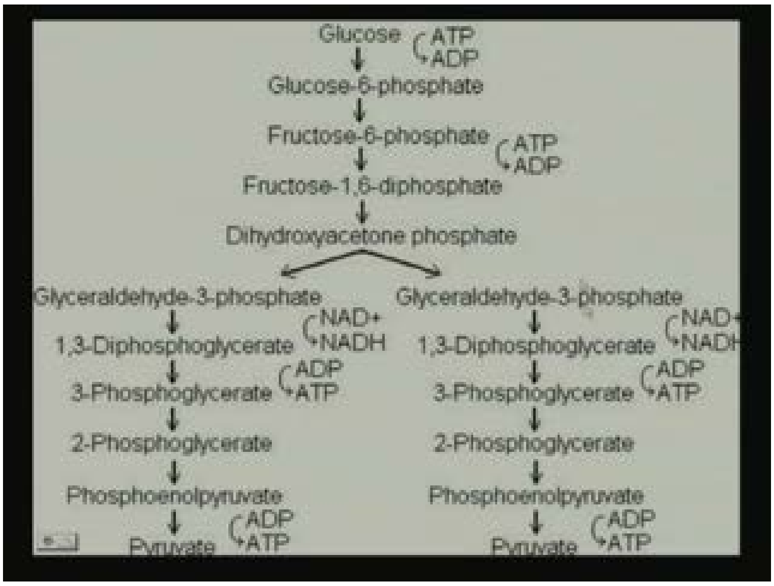

Glucose ATP → ADP
Glucose-6-phosphate
Fructose-6-phosphate ATP → ADP
Fructose-1,6-diphosphate
Dihydroxyacetone phosphate

Glyceraldehyde-3-phosphate
1,3-Diphosphoglycerate NAD+ → NADH
3-Phosphoglycerate ADP → ATP
2-Phosphoglycerate
Phosphoenolpyruvate
Pyruvate ADP → ATP

Glyceraldehyde-3-phosphate
1,3-Diphosphoglycerate NAD → NADH
3-Phosphoglycerate ADP → ATP
2-Phosphoglycerate
Phosphoenolpyruvate
Pyruvate ADP → ATP

Now, if we see the overall reaction then we can find that, first in this step glucose is converted to glucose 6-phosphate, 1 molecule of ATP is utilized glucose 6-phosphate of glucose 1, 6-diphosphate here also, 1 ATP molecule is utilized. But, here in glyceraldehyde 3-phosphate you see, 2 molecule of NAD is produced NADH and 1, 3-diphosphoglycerate is produced that 2 molecules of ATP, and here also 2 molecules of ATP is produced as a result of this reaction.

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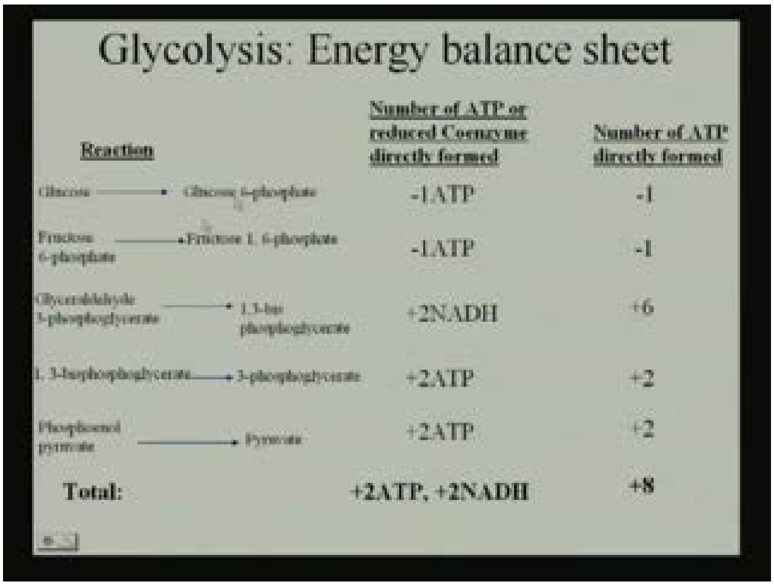

Glycolysis: Energy balance sheet

Reaction	Number of ATP or reduced Coenzyme directly formed	Number of ATP directly formed
Glucose → Glucose 6-phosphate	-1ATP	-1
Fructose 6-phosphate → Fructose 1, 6-phosphate	-1ATP	-1
Glyceraldehyde 3-phosphoglycerate → 1,3-bis phosphoglycerate	+2NADH	+6
1, 3-bisphosphoglycerate → 3-phosphoglycerate	+2ATP	+2
Phosphoenol pyruvate → Pyruvate	+2ATP	+2
Total:	**+2ATP, +2NADH**	**+8**

So, if we see the overall energy balance in this particular reaction, then we can find that this is the utilization of 1 ATP and up to glucose to glucose 6-phosphate, fructose 6-phosphate to fructose 1, 6-diphosphate and in this hence, forth reaction glyceraldehyde 3- glyceraldehyde 3-phosphoglycerate to 1, 3-bisphosphoglycerate, 2 molecule of NADH here 1, 3-bisphosphoglcerate to 3-phosphoglycerate, here also 2 molecule of ATP and phosphoenolpyruvate to pyruvate here also 2 molecule of ATP, is produced. Now, as we know that 1 molecule of NADH is giving 3 molecules of ATP, so we can say that it is approximately the 6 ATP molecules and here 2 ATP molecules, 2 are getting cancelled, so net ATP molecule gain in this fructose is 8.

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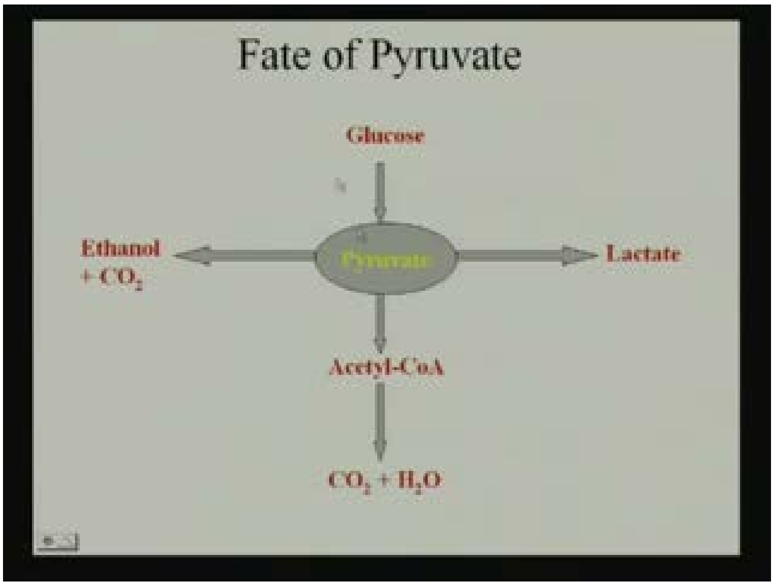

Fate of Pyruvate

Glucose

Pyruvate

Ethanol + CO_2

Lactate

Acetyl-CoA

$CO_2 + H_2O$

Now, once this pyruvate is formed, this pyruvate is being utilized by vary many reactions it can be converted to lactate when there is a (()) of oxygen in the in the muscle or in the cell, under anaerobic condition, it can also produce ethanol and under aerobic condition pyruvate is getting converted to acetyl-coa, and this acetyl-coa is entering to the kreb cycle and what I will be discussing in the next class. And I think today, I have try to give you the overall bioenergetic, the energy utilisation process in the living system, and how the metabolic reactions are taking place within the living system and with this today, I am concluding, thank you very much.