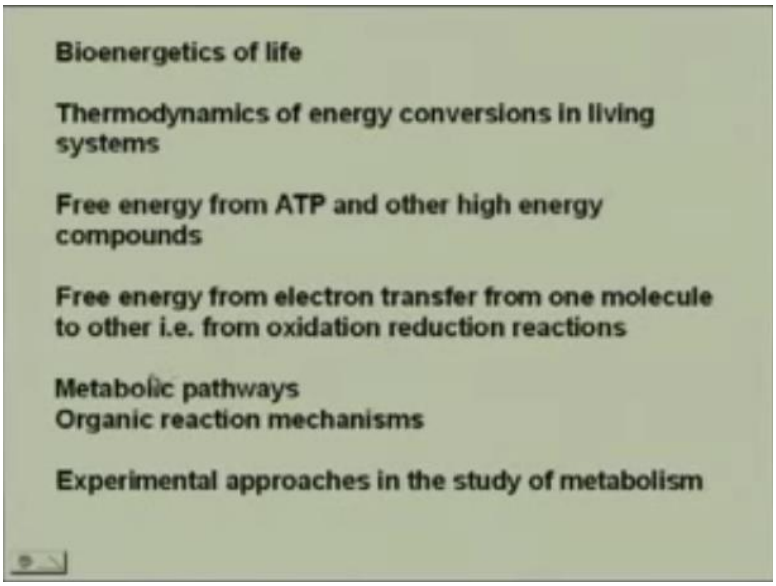
Biochemistry
Prof. S. Dasgupta
Department of Chemistry
Indian Institute of Technology – Kharagpur

Lecture - 23
Bioenergetics - I

We begin our lecture on bioenergetics, which is the final part of this course and we will be speaking of certain aspects of the energy of systems and mostly later on the metabolism of carbohydrates.

(Refer Slide Time: 00:57)

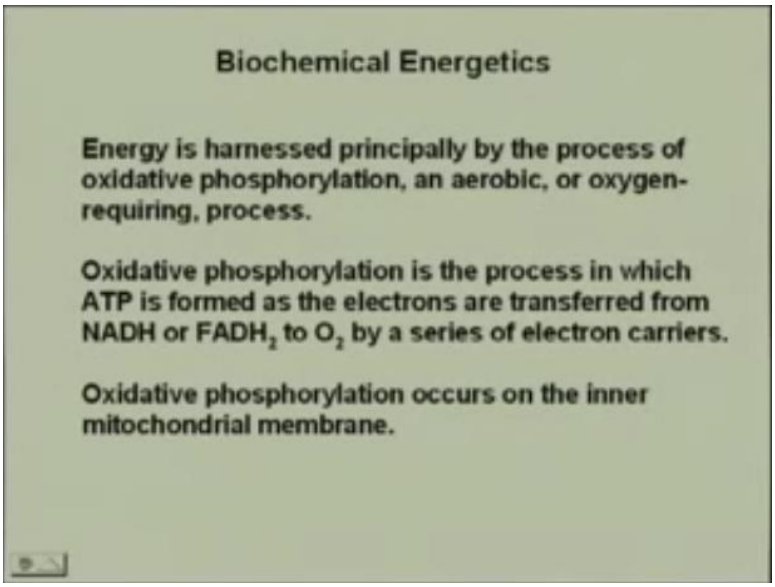


Now, if we consider the bioenergetics of life, we consider the thermodynamics of energy conversions in living systems and we have seen before, how we look at ATP as a source of energy and it will be more apparent now, when we see how this energy of the breakage of the high energy phosphate bond is actually going to give us a lot of the energy that is required to drive these processes.

So, we have the free energy from ATP and other high energy compounds, then the free energy from electron transfer from one molecule to other in ordinary oxidation reduction reactions, where again we will be using some compounds that we have considered that have been derived from the vitamins. Then we will be looking at some metabolic pathways and involved in that we will be looking mostly at the breakdown of glucose, how glucose is broken down in the body and in that there will be certain, not very many, but some organic reaction mechanisms and there will be some studies based on how the energy is utilized in the

processes, in glycolysis or the tricarboxylic acid cycle, where we have the final breakdown of glucose.

(Refer Slide Time: 02:20)

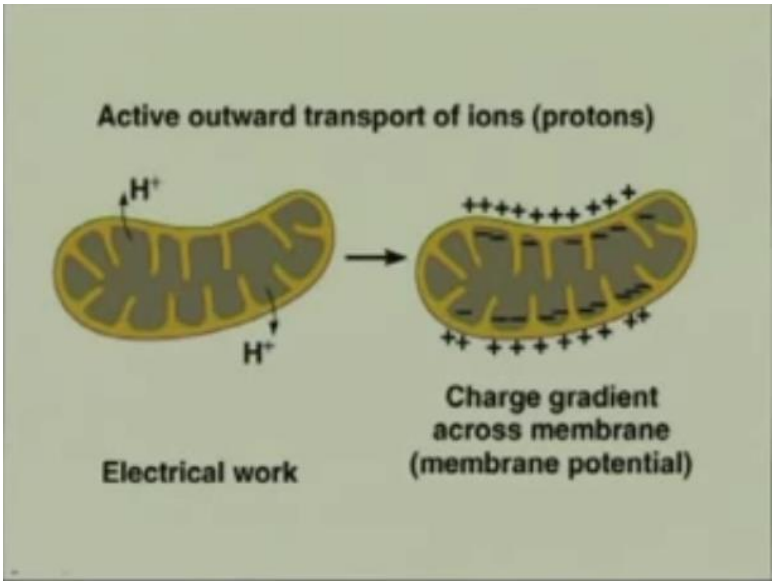


Now, when we consider biochemical energetics, usually the energy is actually harnessed by a process that is called oxidative phosphorylation. This is an aerobic process. An aerobic process means it is a process that requires oxygen. This oxidative phosphorylation is the process by which ATP is formed and you realize that since ATP is the currency of energy, we require its formation at a very high level as well, because its breakdown is going to finally drive a lot of other processes that are going on.

So, the formation of ATP is extremely important and oxidative phosphorylation is the process in which it is formed as electrons are transferred from NADH or FADH2 to oxygen by a series of electron carriers. Now, we won't go into the details of all the mechanism, but we will just look at the broad overview of how this oxidative phosphorylation system works and how actually ATP is formed.

Now, this process of oxidative phosphorylation occurs in the mitochondrial membrane. You have all heard or studied from your school days that the mitochondria is the power house. And what we look at it now, is a bit more detail in the fact that it is the power house because it gets you the source of energy that is ATP.

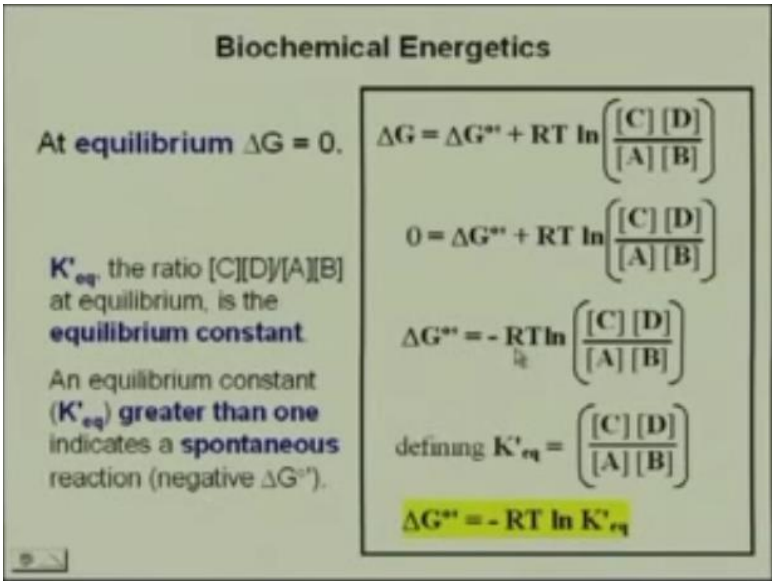
(Refer Slide Time: 03:58)



In a mitochondrial membrane, if we look at the structure of mitochondria, we have this mitochondrial system. This is you know a picture of mitochondria and what we have here is, we have the membrane. We have a charge across this membrane and in here if you remember these folds are called cristae. These folds, actually there is this inter membrane space and there is the inter cellular space.

The cytoplasmic space, the inter membrane space and the outside of the membrane. So, there are these 3 features that we are going to consider in the charge gradient across the membrane and we will see how important that is in driving what is called a proton pump, because this proton pump is essential for the formation of ATP, which occurs in the inner mitochondrial membrane. But before we get into that, we will just look at the basic aspects of energy equilibrium and how they are related in other terms like delta G and all that.

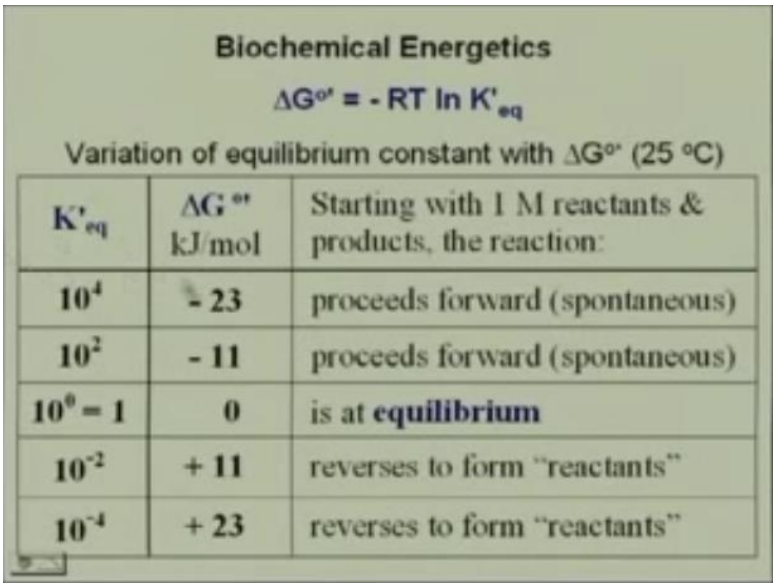
(Refer Slide Time: 05:08)



This is something you have studied before, but in general, when we consider any energetics or any equilibrium, we look at a delta G factor and we know that at equilibrium this value is 0. We have here certain products and certain reactants. A+B going to C+D at a definite temperature and associated with that we can get a specific equilibrium constant and with the equilibrium constant we know that when we have a negative value for the delta G zero prime, you are going to have a spontaneous reaction.

Now, the prime usually refers to, if not mentioned otherwise, it refers to a biological system where the temperature is 37 ˚C. It is not your normal delta G zero where the temperature is 25 ˚C. So if not mentioned the delta G zero prime T has to be 37 ˚C. Now if we look at the variations, if we just consider, you can see how different the variations get.

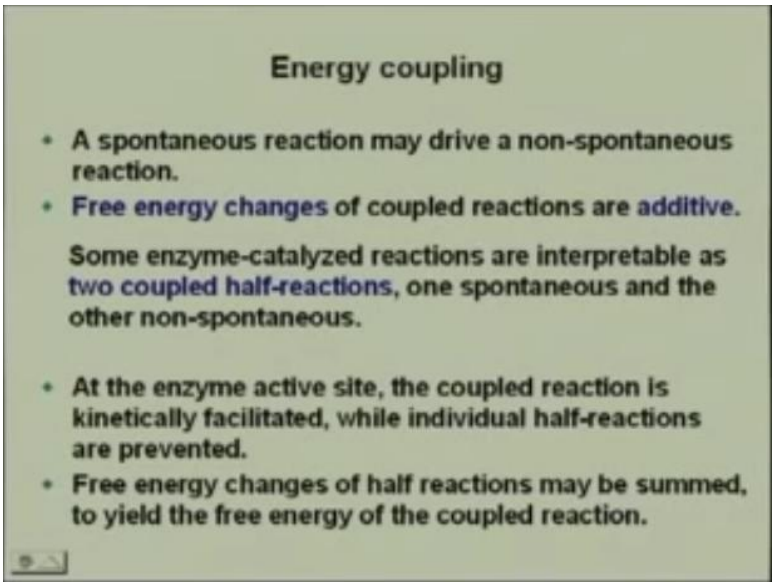
(Refer Slide Time: 06:18)



Now, the calculations here have been done at 25 ˚C for the delta G zero prime, but in normal cases when the temperature is not mentioned it means you use 37 ˚C for your calculation. Now, if we just consider just 1 M concentration of reactants and products, what you will see here is how the order of the equilibrium constant changes more than a 100-fold for relatively smaller value of kJ/mol delta G changes. Now this you have to be very careful about, this is due to the ln feature that you have here.

Since, it is an exponential dependence, what you have is, even if you have just a 12 kJ/mol difference here, you are looking at a 100-fold difference in your equilibrium constant, which means your reaction is going to get to equilibrium at a much different rate than obviously if you had just your energy at this format. Now, when you consider the delta G zero prime values here, you understand that this means you are going to have a forward spontaneous reaction, this is at equilibrium and this goes in the backward direction, a non-spontaneous reaction.

(Refer Slide Time: 07:47)

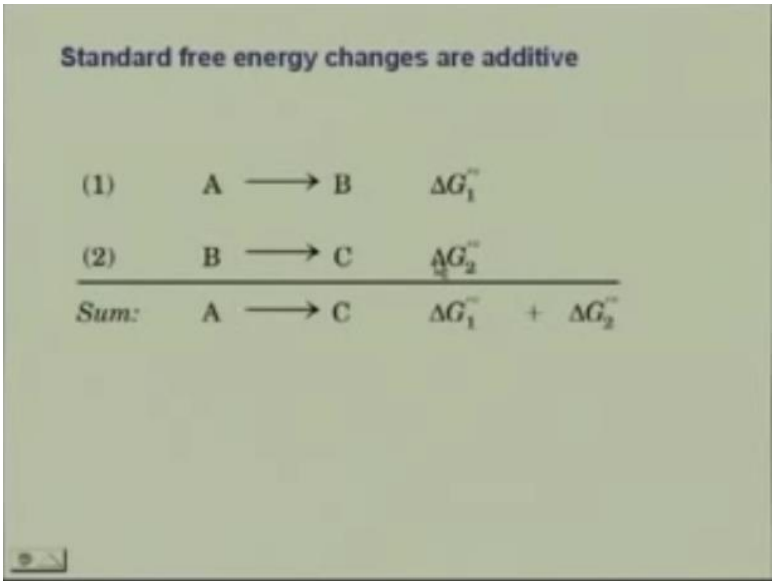


Now, when we consider the energetics, bioenergetics, requires energy coupling. You do not have a continuous spontaneous reaction usually, because that is going to form a large amount of products. You might not need all those products all the time. So the process, the whole process of the formation of the reactants, of the products from the breakdown of the reactants in the enzymatic processes has to be tightly regulated, because you understand, remember we studied feedback inhibition.

What was feedback inhibition? It was where you had your final product inhibit the initial enzyme. So what you are doing here in this case is, you have a spontaneous reaction that drives a non-spontaneous reaction. The energy is coupled in such a way that the free energy changes of these reactions are additive. And what we have is these enzyme catalyzed reactions they are interpreted as two coupled half-reactions, where you have the energy of one compensate the energy of the other, more than compensate it, where you are going to get a value that is going to be making the specific reaction spontaneous in nature.

Now what happens is, at the enzyme active site the coupled reactions are kinetically facilitated, which means that they go at a specific rate, but the individual half-reactions are prevented. You do not have just a certain half-reaction going on, but when the enzyme active site has both the reactants, there is a coupled reaction that goes on and the free energy changes of the half-reactions are summed to yield the free energy of the coupled reaction, which usually gets to a spontaneous reaction, a negative value. We will see, I have an example here.

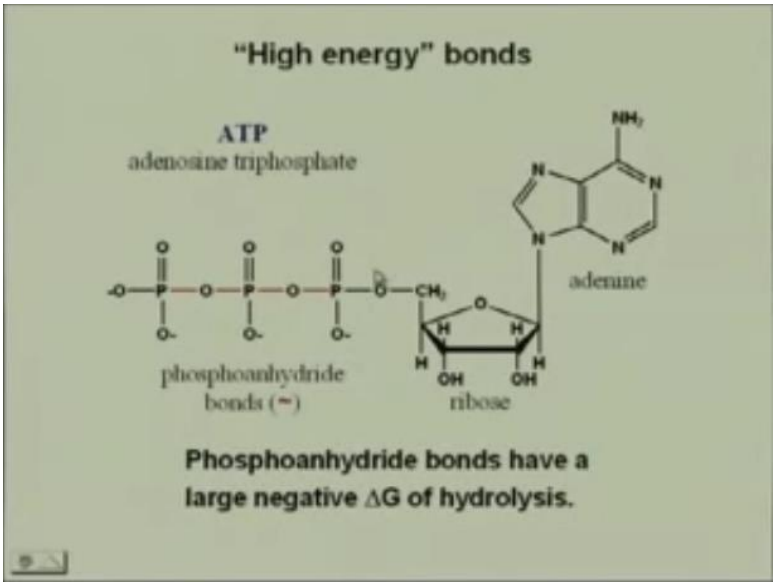
(Refer Slide Time: 09:47)



So essentially what we are looking at is, we are looking at a standard free energy change here. So if we have half-reaction that goes from A to B and another half-reaction that goes from B to C, then, we can have the overall reaction go from A to C and the standard free energies of both these are going to be additive. Now, it may so happen that you do not have the B component present in both cases.

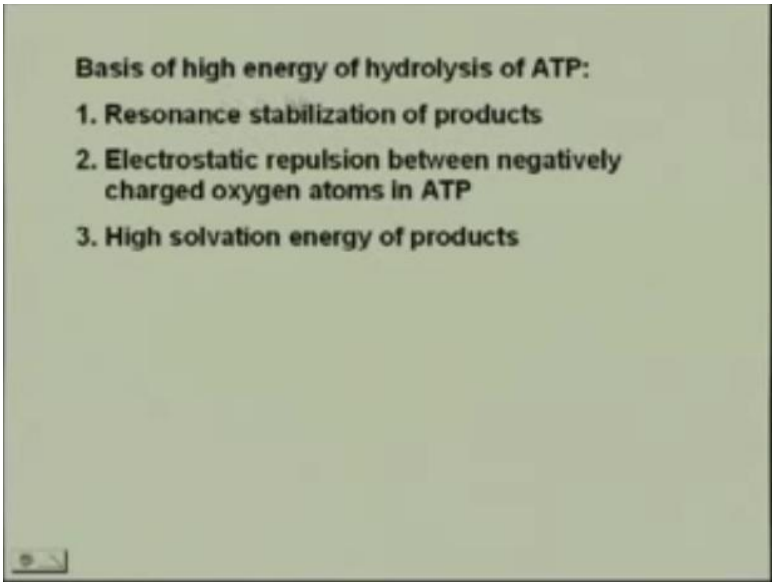
That is one such case. Another case may be you have a completely different reaction that is actually going to provide the energy for this reaction to go forward. That would be a normal biochemical coupled reaction and in most cases, we get the energy from the ATP breakdown.

(Refer Slide Time: 10:36)



So, what are we speaking about? We are speaking about this high energy bonds of ATP. This is the structure that you now recognize. And, what we have here is, we have a very large negative delta G of hydrolysis meaning that this breakdown is extremely spontaneous in nature.

(Refer Slide Time: 10:54)

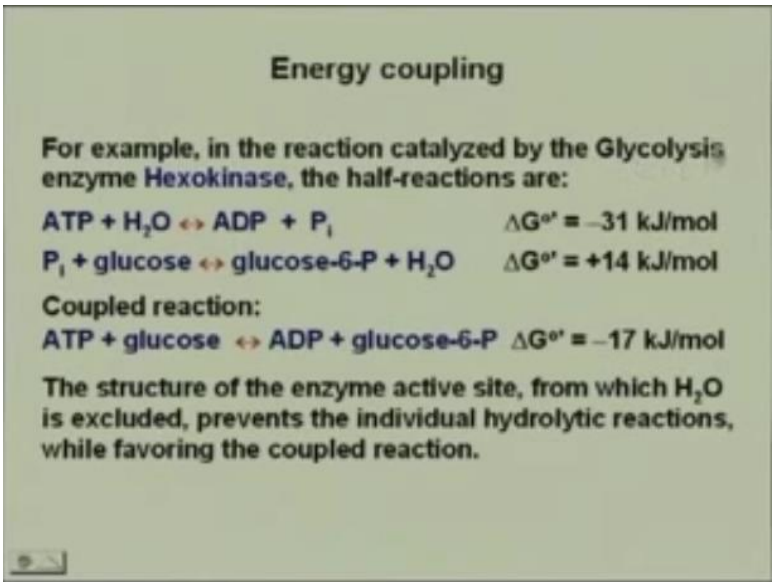


Now, the basis of the high energy of hydrolysis of ATP is due to the resonance stabilization of the products that you get. It is also due to the electrostatic repulsion between the negatively charged oxygen atoms in ATP. Where are these negatively charged oxygen atoms? They are

here. So, you would not have them one beside the other all the time, so it would be relatively easier for it to break off to give you ADP+Pi or AMP+PPi.

And also we have a high solvation energy of the products, which amounts to the high energy of hydrolysis for ATP. Now, when we have the reactions that require the breakdown of ATP are such reactions that are going to have a positive delta G by themselves. So, if we look at such an example.

(Refer Slide Time: 11:54)



For example, the enzyme hexokinase, a kinase is a transferase enzyme that transfers a phosphate group. It does not usually get the name, it is a transferase but a kinase is a specific type of transferase that transfers the phosphate group. Now, in the reaction catalyzed by the process glycolysis, which we will be studying in detail, when we go to the glucose breakdown, the first step in the glucose breakdown is the formation of glucose-6-phosphate.

Now, the formation of glucose-6-phosphate from glucose + Pi is a plus value of 14 kJ/mol. So, by itself it is non-spontaneous. So, you will not have your glucose with the help of the enzyme hexokinase, now this name hexo means it is working on a 6 membered carbon ring or your 6 membered glucose carbon sugar here. So it is a hexose, it is the kinase working on a hexose, so it is a hexokinase which means that it is going to be involved in the transfer of a phosphate ion, from just the Pi in this case, to glucose giving you glucose-6-phosphate.

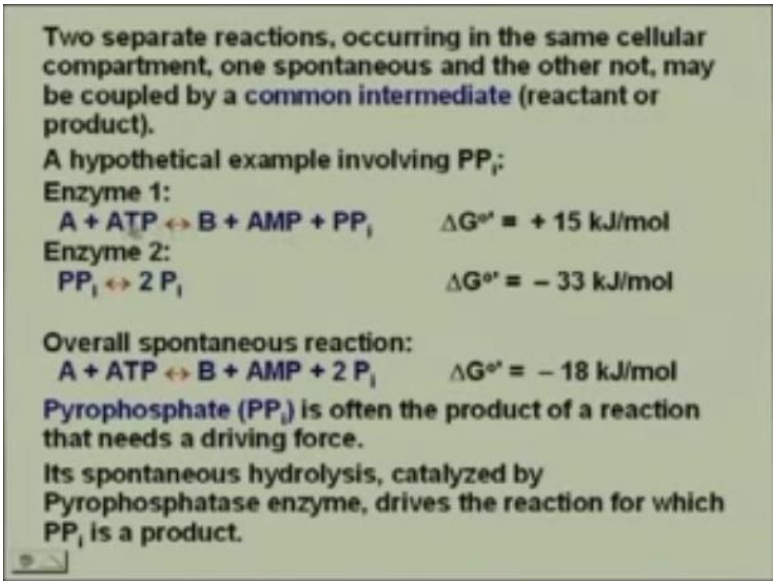
Now, as I just mentioned, this overall reaction has a positive delta G zero prime. However, if you couple it with the hydrolysis of ATP, ATP hydrolysis gives you ADP+Pi and we have a

very large negative value here for the breakdown of ATP. And when you couple these 2 reactions together what is happening is, you have ATP + glucose form ADP + glucose-6-phosphate giving you an overall favorable delta G zero prime of the reaction. So, this makes this reaction spontaneous. So, this is what you would call energy coupling.

And the structure of the enzyme active site, from which H2O is excluded, prevents individual hydrolytic reactions but it does favor the coupled reaction. You understand that the enzyme active sites are extremely specific in the way they work, you have seen some enzyme active sites and how they actually work. Hexokinase, we will not go into the details of how the hexokinase works, but for understanding the energetics what you have to realize here is that the non-spontaneous reactions are coupled with other reactions that are spontaneous in nature, in this case ATP hydrolysis, but this together will give you a favorable energy, which makes the reaction go forward.

Now, nature has chosen certain specific hydrolysis reactions for the specific types of non-spontaneous reactions. For example, in this case we need ATP hydrolysis, but in some cases if the energy is enough to be compensated by another specific hydrolysis or another electron carrier or whatever, then it may not require this amount of energy for the couple reaction and then what is chosen? Another hydrolysis is chosen to couple it so that we do not have too much extra energy.

(Refer Slide Time: 15:48)



Now, we can have this also. This is another case, where we have 2 separate reactions that occur in the same cellular compartment. One is spontaneous and the other is not. That would

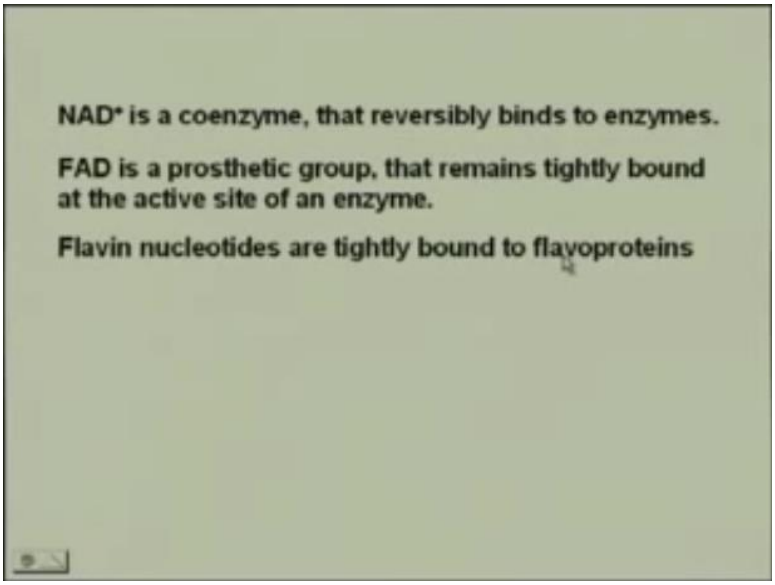
be typical of a coupled reaction and it is coupled by a common intermediate. For example, if we look at a hypothetical example that involves PPi, we have A+ATP going to B+AMP+PPi. Now, this particular reaction that occurs in enzyme one has a positive delta G.

Now, it having a positive delta G means it is non-spontaneous. So, if we want to form B from A, we have to have a corresponding compensatory reaction that is going to have a delta G that is negative. Then, we can couple it with this reaction to give an overall spontaneous reaction and the energy has to be such that it has to be obviously, the negative value has to be obviously more than this in magnitude.

So if we look at enzyme 2 that is breaking up the PPi into 2 Pi, it gives us a delta G zero prime of -33 kJ/mol, which more than compensates for the spontaneity of this one, so the overall spontaneous reaction is going to give us A+ATP and B+AMP+2 Pi. Again, we are actually looking at an ATP breakdown, but we are not going to ADP+Pi. In this case, the first reaction itself is forming ATP is breaking down into AMP+PPi and the reaction which couples or which actually provides the energy for the first reaction to go forward is the PPi breaking down into 2 Pi.

So, what happens is, this pyrophosphate is often the product of a reaction that needs a driving force. And, we have this breakdown. Now, if you look at both the examples that I showed you, both of them are breaking down ATP. Which means that ATP has to be produced somewhere. If you don't have enough production of ATP obviously none of these reactions are going to be possible.

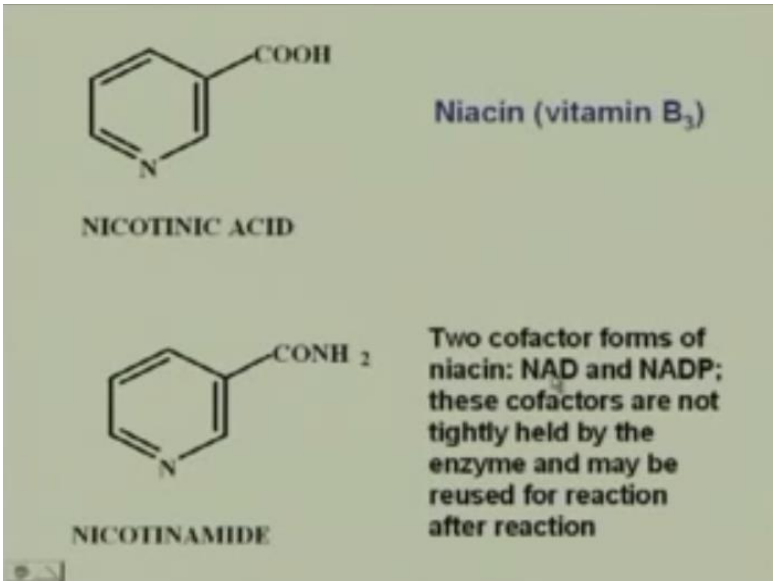
(Refer Slide Time: 18:25)



So, we are going to look at generally how ATP is formed in a very simplistic manner. Now, we have looked at these molecules before. We will go into some of the details of what we looked at before. We considered these when we studied vitamins and coenzymes. NAD+ is a coenzyme that reversibly binds to enzymes. That is what a coenzyme is and FAD is a prosthetic group that remains tightly bound to the active site of an enzyme.

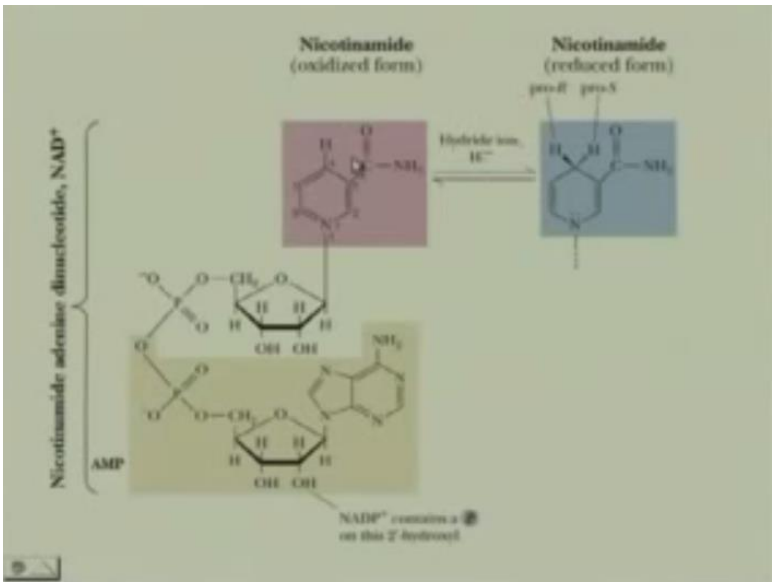
And we will see how these are utilized basically in the process of oxidative phosphorylation to actually give you ATP production. And these flavin, we will look at what these molecules are. The flavin nucleotides are tightly bound to what are called flavoproteins. So, the proteins that have the FAD or the flavin nucleotides are called flavoproteins and these are required in the reactions of oxidative phosphorylation to give you your enough energetics or whatever is required for the production of ATP.

(Refer Slide Time: 19:42)



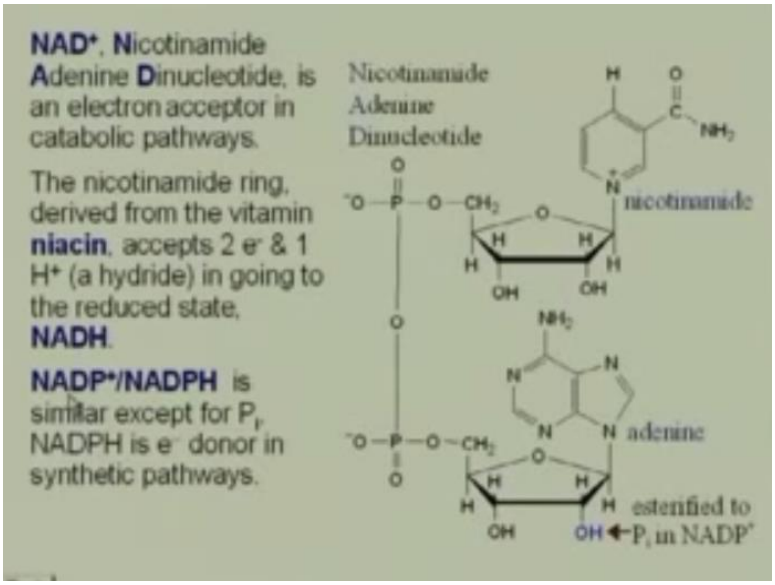
Now, this is something that we studied before where we are looking at vitamin B3, niacin, here where we have 2 cofactor forms that were NAD and NADP. They are not tightly held. They are the cofactors and they are reused for reaction after reaction. We do not use them in the raw form here.

(Refer Slide Time: 20:09)



What we have to transform them is to NADP plus or NADP. Now, what happens in these reactions is we have an oxidized form and a reduced form.

(Refer Slide Time: 20:25)

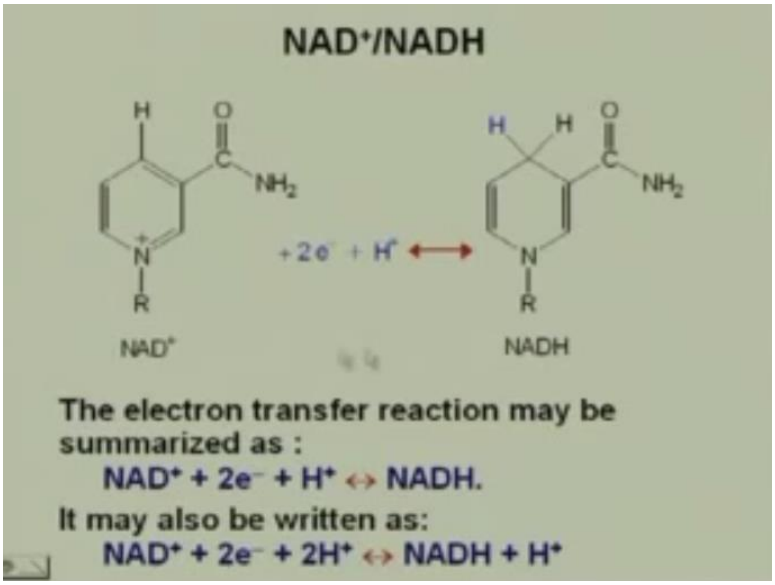


What are these forms? When we are looking at nicotinamide adenine dinucleotide, we know that we have the nicotinamide adenine dinucleotide. A nucleotide has a single phosphate. A dinucleotide has these two phosphates and we have a nicotinamide, an adenine and a dinucleotide. So, we have NAD. It is an electron acceptor. We will see what changes, because the rest of the molecule is not going to be required. The only difference in NA, this is NAD plus, this nitrogen has a plus, this particular nitrogen has a positive charge to it.

The only difference that we have between NAD plus and NAD plus is this two prime OH on the adenine nucleotide is phosphorylated. So, we had NAD plus. So, we have NAD plus here, this is NADP plus sorry. We have NAD plus, where we have the OH here. We have NADP plus, when we have the phosphate here. Now, we have therefore the features of NAD plus or NADP plus that we have the nicotinamide that has derived from niacin.

So, this is derived from the vitamin B3 niacin. It accepts two electrons and one proton. That means a hydride and goes to a reduced state NADH. Similarly, we have NADP plus and NADPH. And, it is similar as I said, it only has the extra phosphate at the two-prime position.

(Refer Slide Time: 22:28)



So the variations that you are looking at is NAD plus and NADH. So if we look at the previous slide. The rest of this molecule is required for recognition for the enzymatic process but for the NAD plus going to NADH, it is only this part, that is the nicotinamide part that is required, so we refer to the rest of the whole portion R, nothing else but R. That is exactly what we have here. So, R is the rest of the NAD plus. So, we have the N plus here. Now, what it does? Accept its two electrons and a proton.

This becomes, it loses its plus, it has an additional H, and so it is now NADH without the positive charge. So, what it accepts is two electrons and a proton. The electron transfer reaction can be summarized as NAD plus + two electrons + H plus that is going to NADH or it is also written as NAD plus + two electrons + two protons going to NADH + H plus.

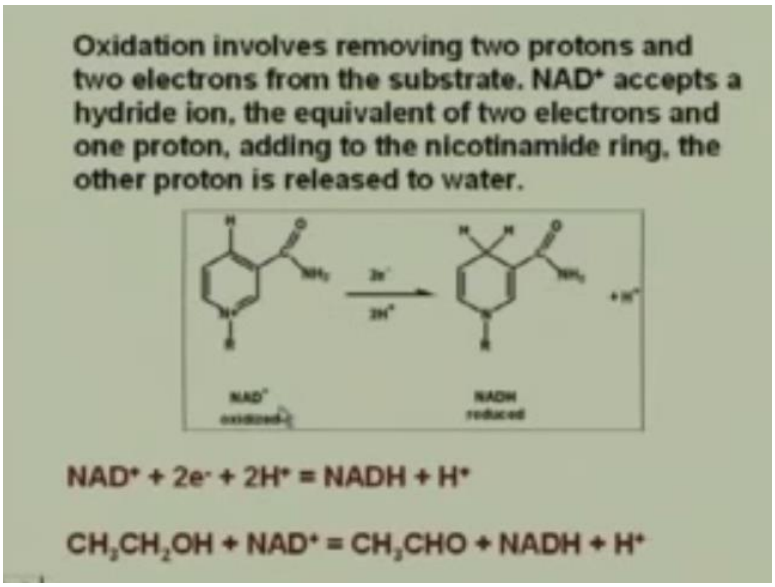
Now, you have to recognize here that the reactions that NAD plus or for example, when we do, I will just show you, when we do FMN and FAD as well, these reactions are going to occur in these cofactors of prosthetic groups are going to be required in enzymatic reactions that are going to be of what type? Of redox type. Because, either the hydrogen has to be taken away or the hydrogen has to supplied. So, in that case, we cannot have the ATP come into the picture.

So, when it is a certain dehydrogenes enzyme or an oxidized type of enzyme, it will require NAD plus or FMN or FAD for the particular reaction to go forward. So, you have to recognize in the energetic procedure what sort of a transformation is taking place. Because, each of these are transformation steps, break down steps that we are going to study and as we

go through them, we will see that obviously when you want to add a phosphate with the help of a kinase that is going to transfer the phosphate you cannot use any of these, you have to use ATP.

But, when we have a redox reaction that is going to use, redox reaction that is dehydrogenase or an oxidase you will require NAD plus and NADH and depending on the enzyme that you have, you will either use this or we will use FMN and FAD.

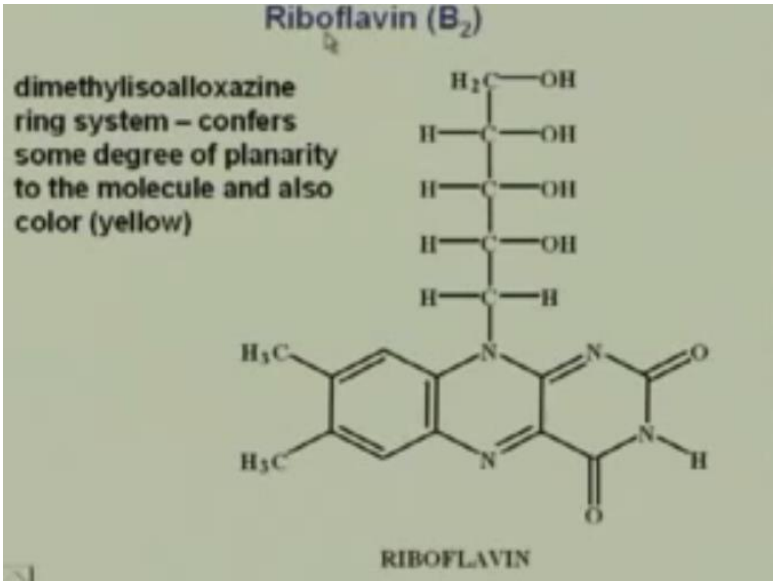
(Refer Slide Time: 25:22)



So, basically what we have? This is one example where we have ethyl alcohol going to acetaldehyde, where we are looking at NAD plus, this going to NADH + H plus. Basically, what happens is, the oxidation involves removing two protons and two electrons from the substrate NAD plus. So what is here, your substrate is going to the product, where you are moving two protons and two electrons from the substrate and where this is going? This is going to NAD plus to form NADH.

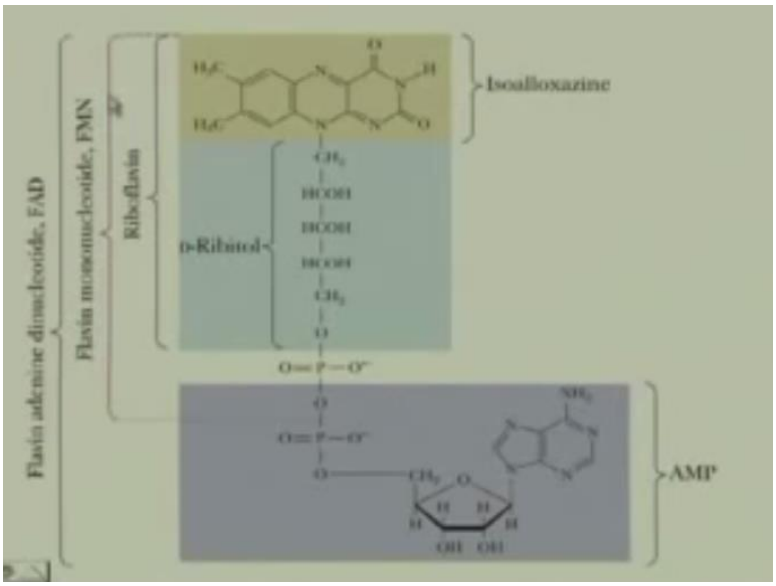
NAD plus is taking up these two protons and two electrons. It accepts a hydride ion that is the equivalent of two electrons and a proton. It adds this to the nicotinamide ring and the additional proton is released to water. So, basically any reaction that is going to require the removal of two protons is going to use NAD plus. NAD plus is going to take up those two protons. That is as simple as how it actually works.

(Refer Slide Time: 26:30)



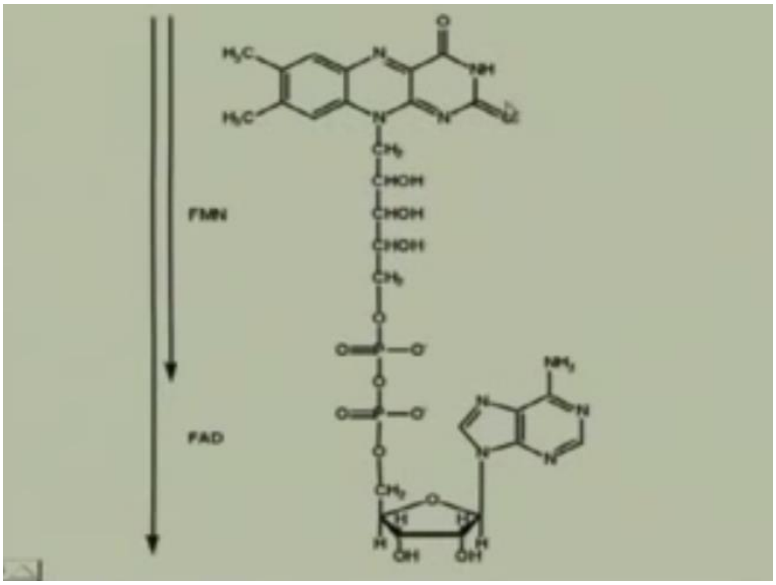
The other one that we are going to be using in oxidative phosphorylation for FAD or FMN is derived from this vitamin Riboflavin, vitamin B2.

(Refer Slide Time: 26:46)



And the structure there is a Flavin mononucleotide, where what we have is, we basically have this isoalloxazine ring. This ring, isoallaxozine ring is what is required here and what is going to be taking up the hydrogens.

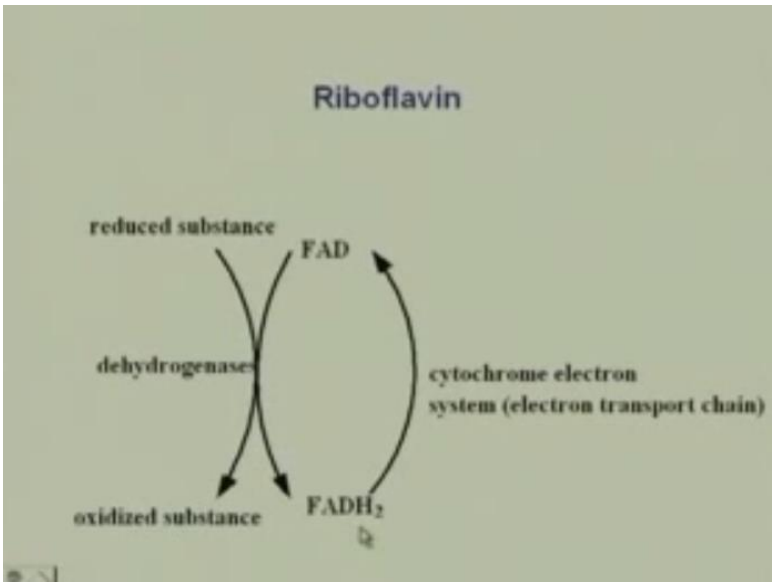
(Refer Slide Time: 27:13)



So, again when we are looking at it, this is Flavin mononucleotide up till this part. Why mononucleotide? Because, we are talking of one phosphate. When we have Flavin adenine dinucleotide, we have the adenine here we have the other phosphate here. So, we have FMN or FAD. So, but each of these we are going to refer to all of this portion as R.

Now, then depending on the energetic of the process, depending on what is utilized or what sort of whether it is FMN attached to the enzyme or the FAD attached to the enzyme, the reaction will proceed accordingly. But, basically what is going to happen is this ring is going to do what? Take up the hydrogens.

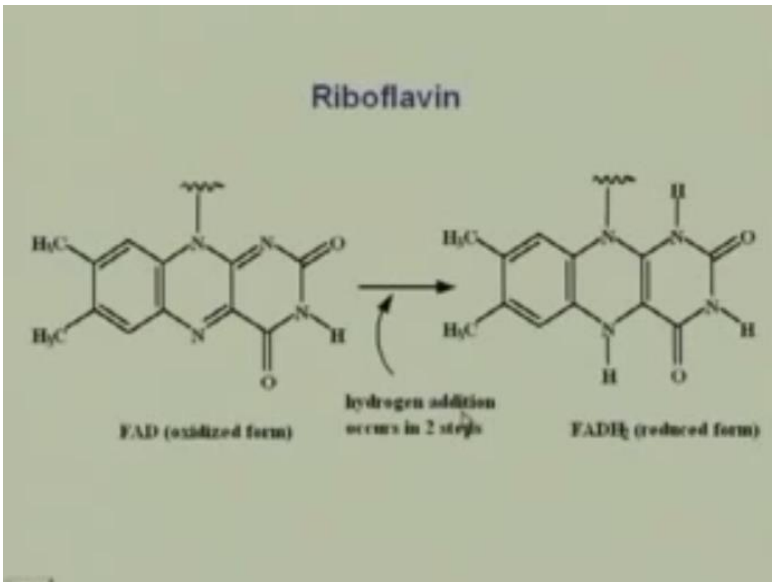
(Refer Slide Time: 28:08)



How is it going to do that? We have a reduced substance. We have FAD. We have a dehydrogenase that takes, so the H2 from the reduced substance is taken up by the FAD.

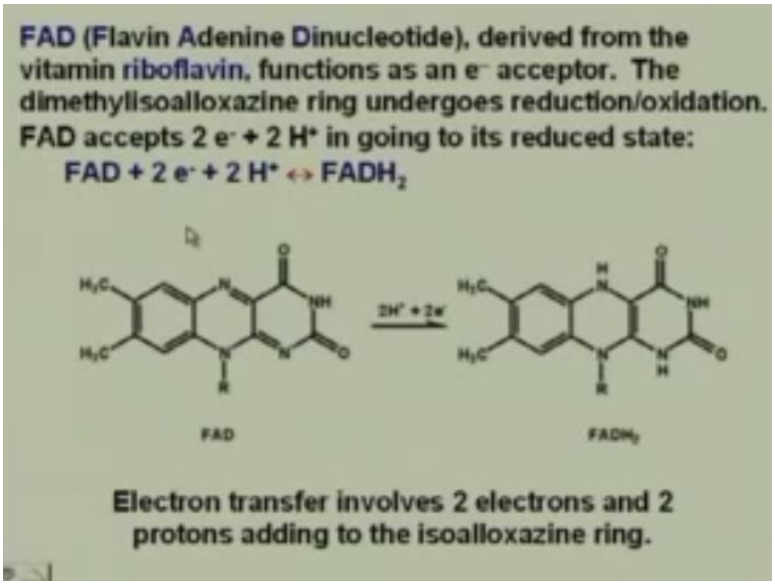
Similar to NAD plus going to NADH. So, we have FAD going to FADH2. This is an example of where we have cytochrome electron system in the electron transport chain. So, we have the reduced substance going to an oxidized substance with the help of an enzyme dehydrogenase in this case that is going to abstract the hydrogens from the substance from your reactant and give it to FADH2, giving to FAD forming FADH2.

(Refer Slide Time: 28:53)



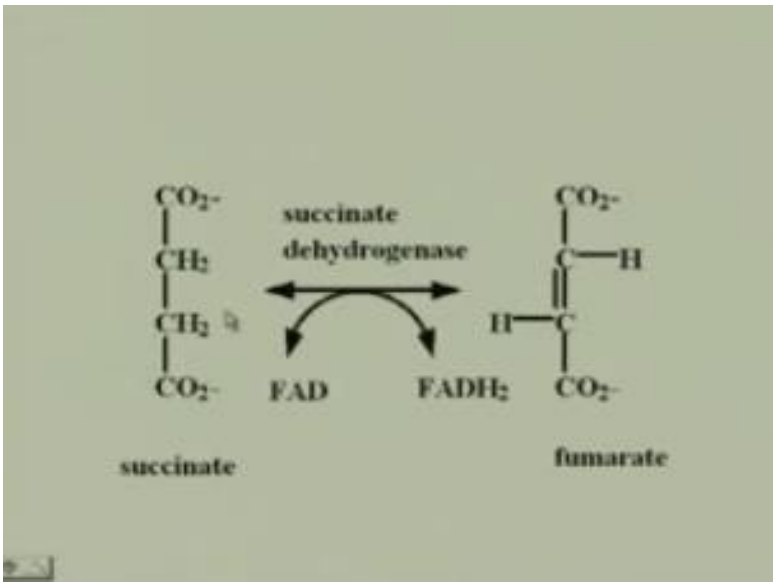
Ok. So, what happens is, we have, this is the rest of the ring you now recognize, this is just the top portion that we are interested in. So, we have FAD. The hydrogens are taken by this nitrogen and this nitrogen. So, we have one here and one up there. So, we have the hydrogen addition in two steps, finally getting from FAD to FADH2.

(Refer Slide Time: 29:23)



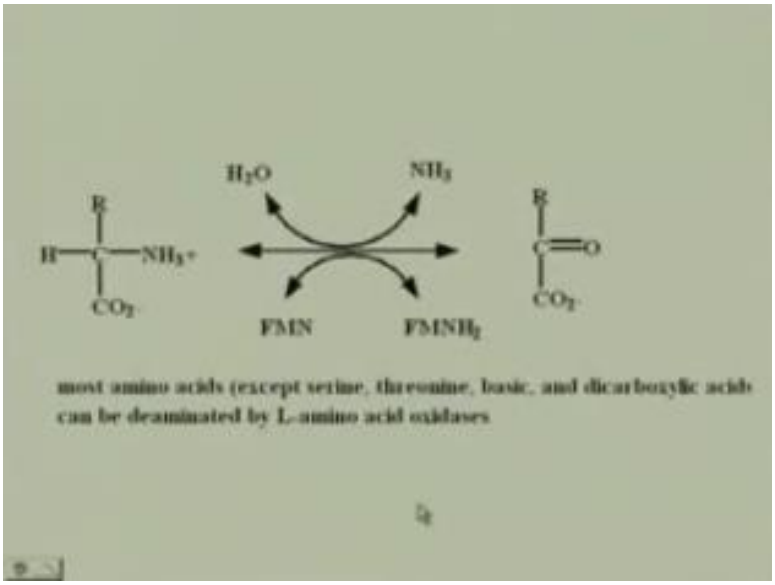
So, what happens is we have our R group here, our FAD and what is going to happen? We have the two protons taken up by the two nitrogens on FAD and it is going to give you FADH2. So we have FAD + two electrons + two protons going to FADH2. And where is it getting these protons from? It is getting them from the certain substrate that has to be converted to the product which will not have the two hydrogens.

(Refer Slide Time: 30:00)



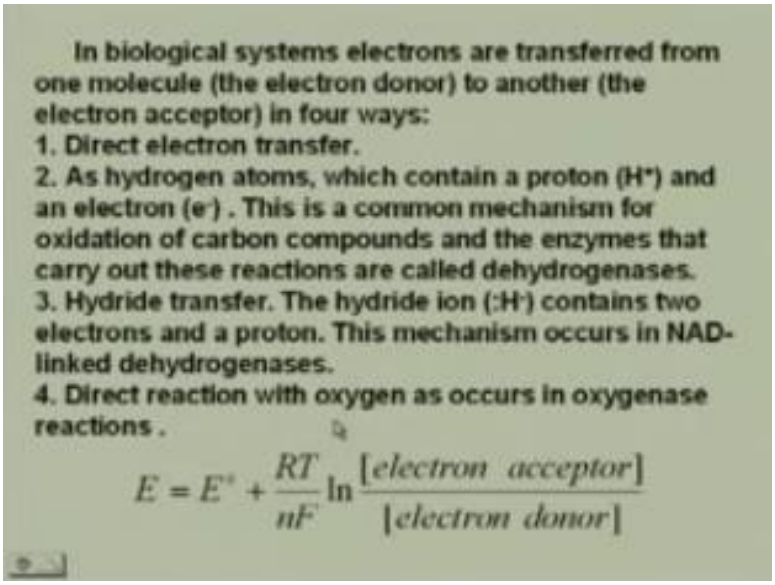
This is an example, where we have succinate, succinate dehydrogenase. So, what is it going to do? Abstract the two hydrogens. In the abstraction, somebody has to take it up. That is simple as that. What is going to take it up? FAD, in this case, is taking it up. And what is happening to FAD? It is forming FADH 2. So, this is also reaction, so succinate dehydrogenase alone will not work. It has to have, first of all, it has to have a place to put these two hydrogens, FAD takes up the two hydrogens forming FADH 2 and in the event, you get your fumarate from succinate.

(Refer Slide Time: 30:46)



So we also have FMN, going to FMNH 2. Here, you are having a release of your NH 3.

(Refer Slide Time: 31:01)



So basically the reactions are going to have proton transfers, electron transfers, in your changes. Now, in these systems, we looked at some examples, where we have NAD plus going to NADH, FAD going to FADH 2 or FMN going to FMNH 2. Now, what is happening in these electron transfer systems, all of you know (()) (31:28) equation. Now, when we consider the biological systems, the electrons are transferred from one molecule to another, just in a normal electron transfer reaction.

But, these can occur actually in four different ways in biological system. So, what are these different ways? We can have direct electron transfer. That is one possibility. We can have them transferred as hydrogen atoms that contain a proton and an electron. And this is a

common mechanism for the oxidation of carbon atom, carbon compounds that we just saw, using enzymes called dehydrogenases.

We can have hydride transfer, the hydride transfer, we saw in the process of NAD, where it takes up the hydride. So the NAD plus goes to NADH. So, we can have direct electron transfer, we can have the transfer as hydrogen atoms, we can have hydride transfer or we can have direct reaction with oxygen. Usually in aerobics systems, where you have oxygen available, you can have direct reaction with oxygen as occurs in certain oxygenase reactions. So, these are the four processes by which we can have electron transfer.

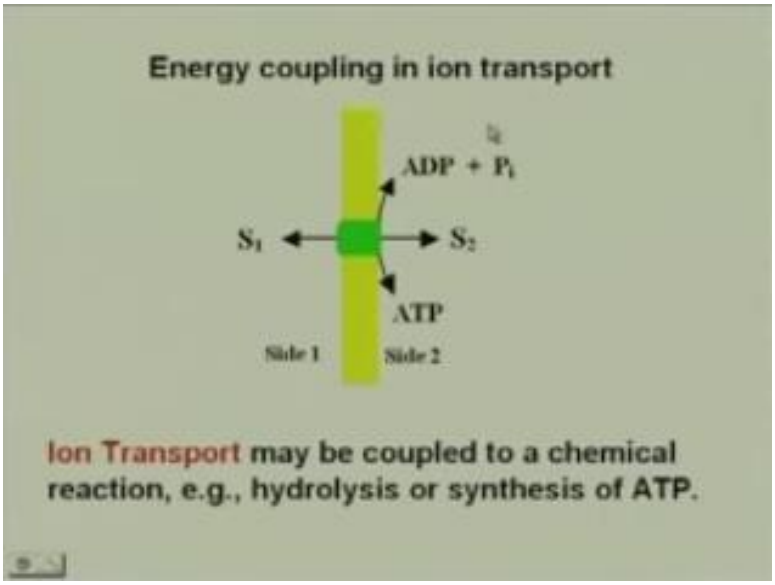
All of these are redox processes. All of these will be using redox enzymes. Redox enzymes are either dehydrogenases or oxidases. So, we can have just direct electron transfer, we can have hydrogen as hydrogen atoms that contain a proton and an electron, we can have hydride transfer or we can have a direct reaction with oxygen, in the presence of oxidases. So, the enzymes that we looking at are dehydrogenases and oxygenases.

So, when we go to the breakdown or the whole metabolism say of carbohydrates, as soon as you look at the reactant and the product, you should first of all be able to identify what is going on. If it is losing hydrogens, then, you know that you have to have, you are having a redox reaction take place. Now, in the process that redox reaction is taking place, the enzyme therefore that you will be using is either a dehydrogenase or some sort of oxidase, a reverse process that is going to happen then.

In that case, you have to have a cofactor that is going to be NAD plus or you have to have the prosthetic group FMN or FAD. So, when you look at these reactions, you have to recognize the type of enzyme that is involved, whether the reaction is of a redox type, whether the reaction is a transferase type, whether the reaction is of isomerization, where the enzyme will be nothing but an isomerase.

When we studied the different processes of metabolism of the carbohydrates, we will see, how the each of these enzymes, we should be able to recognize how each of these enzymes require a specific cofactor or a specific prosthetic group for it to work.

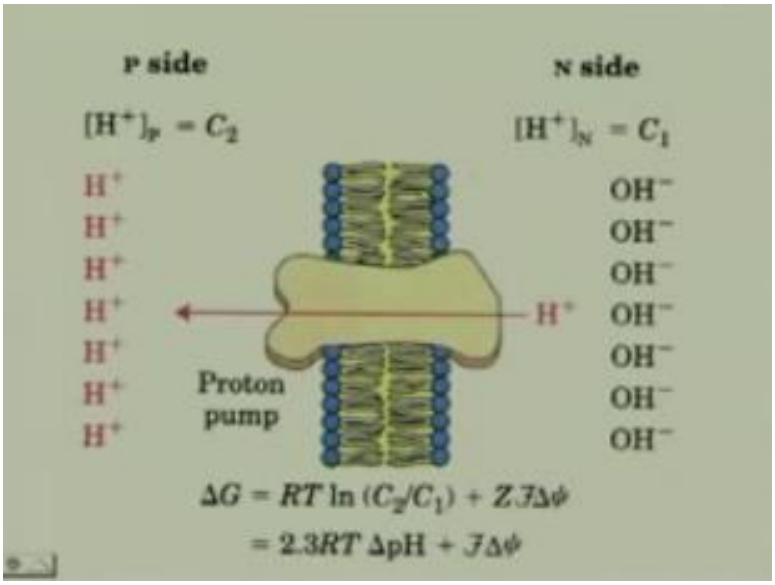
(Refer Slide Time: 34:45)



Now, in the energy coupling in ion transport, we have, say the transfer of S1, S2. In utilizing the breakdown of ATP, to form ADP + P i, we have a couple reaction. So, we are coupling this usually, to a certain chemical reaction, when we require the energy, we are using hydrolysis. We are using hydrolysis of ATP. Why is it so efficient? It is because of the resonant stabilization and certain other factors that I mentioned.

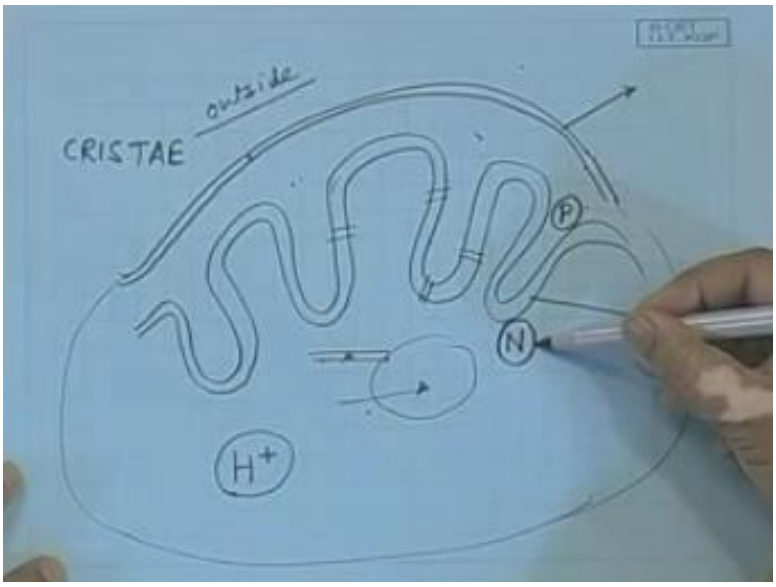
So, the hydrolysis of ATP in the couple reaction is going to give us the possibility of ion transport. But, we also have to remember that we have to produce ATP.

(Refer Slide Time: 35:34)



Now, this is what we have in, you recognize this now as a membrane. And we have a certain enzyme that is going to act as a proton pump. Now, what happens here is, let me just show you the picture,

(Refer Slide Time: 36:00)



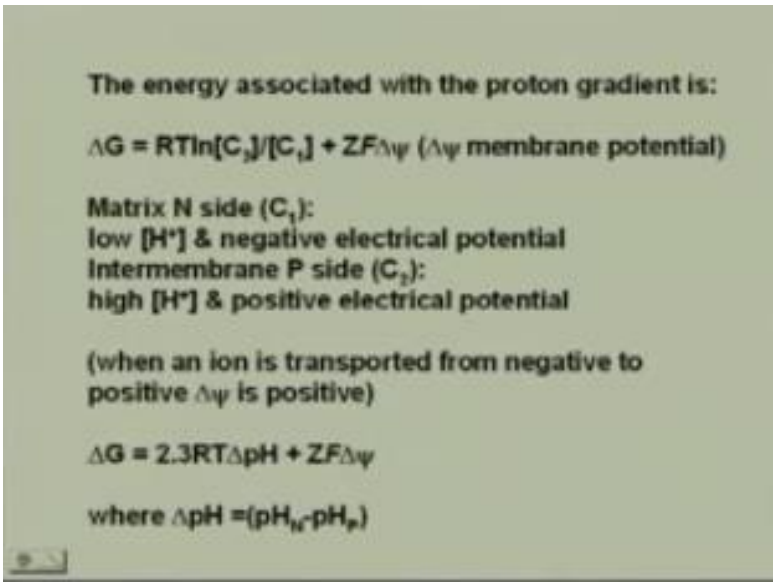
You are looking at, see this is your mitochondria, the outer surface of the mitochondria. So, this is also a membrane. So, we have a lipid bilayer here. We also have an inner membrane that has these folds. So, this is the cross section of your mitochondria. Now, this is also a lipid bilayer. These are folds called cristae. Oxidative for, so what is this? This is the outside of the mitochondria. This is the outer membrane of the mitochondria.

This is the inter membrane space. This is the inner membrane of the mitochondria. So this is the inner membrane, this is the outer membrane and this is the inter membrane space. It is not outside the mitochondria. It is within the mitochondria, but outside the intra, the intracellular space of the mitochondria. It is called the internal matrix rather. So, it is away from this matrix, but also away from the outside of the cell.

It is this area, where, so this is the place, the inter membrane or rather the inner membrane, where the process of oxidative phosphorylation occurs. Now, for the production of ATP, we need H plus. ATP reactions occur in the mitochondria. The reactions that we just mentioned, the couple reactions occur in the mitochondria. So, the ATP has to be present in the matrix of the mitochondria, for the reaction to occur.

What happens therefore is, this H plus is required for the production of ATP. Now, if we go back to the slides here, we have what is called a positive side, that is called the P side and we have a negative side called the N side. Let me just go to the next slide, which is going to be, yes, this one.

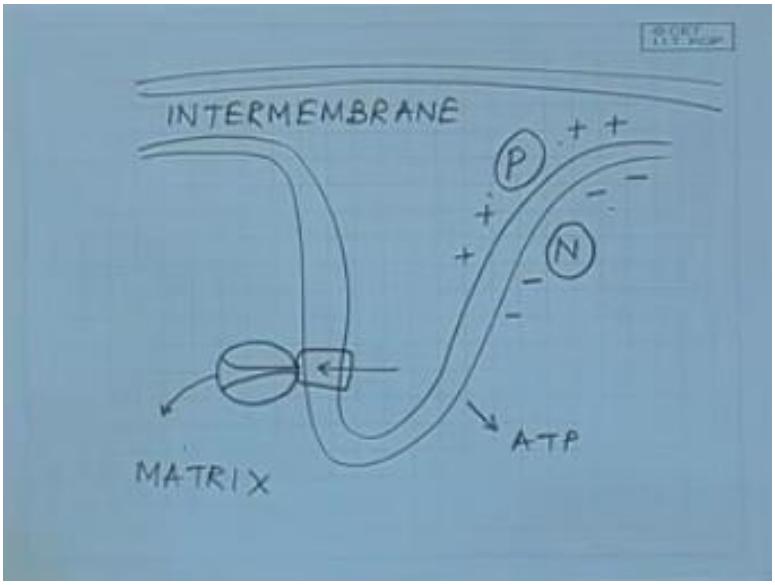
(Refer Slide Time: 38:34)



We have the matrix side, that is the N side, so in my diagram, this is the N side, the matrix. This is the P side that is the positive side. So, what I am going to do now is I am going to blow up a part of this region, so what you have to understand here is, we have the whole mitochondria here, we have the inner folds of the membrane, of the inner membrane, that are called cristae of the mitochondria.

The spelling of cristae is this. We have the cristae of the mitochondria. We have an inter membrane space that is the P space and we have an N space, which is the matrix.

(Refer Slide Time: 39:40)



Now, when we look at, therefore a single fold, so this becomes a single cristae now and we have our outer membrane. So this is my matrix, this is my inter membrane space. What

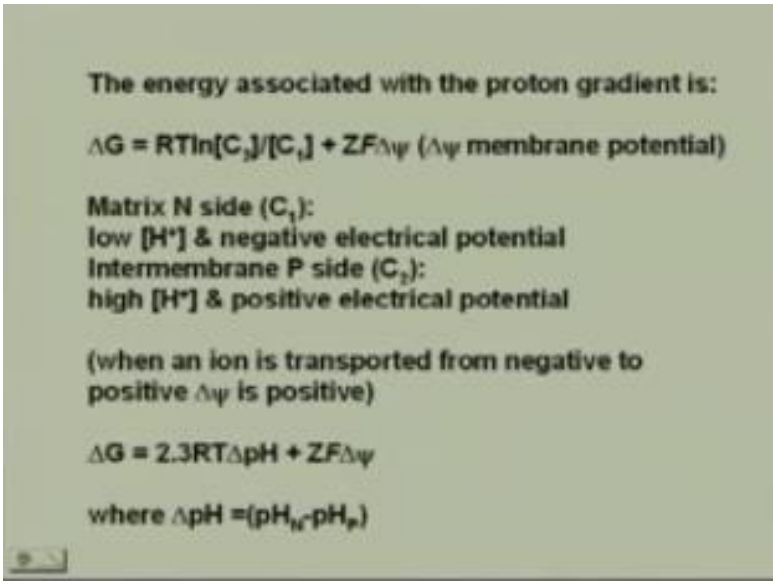
happens here is, here I have a positive charge, which means I have a larger number of protons here. Outside, rather in the matrix, I have what is called the N side. It is very difficult to sort of, mention an inside and an outside here, you understand, because all of these are actually inside, but this is inter membrane and this is matrix side. We have a relatively less part or the N side is more negative.

Now, for the production of ATP, first of all, we have to realize that we need ATP inside the matrix. So, the ATP production has to be inside. But, for the ATP production, we need protons. And the protons are at a higher concentration in the inter membrane space. So what you have to do is, the way the, we have a certain protein that is called ATP synthase, which we will look at the structure in a moment, where we have the protons that have to get in here and ATP is produced here.

The ATP has to be produced in the matrix, because all the reactions are going on in the matrix. But, it requires large amount of protons for it to occur. That means, what has to happen is protons have to be pumped from the inside to the outside, against a proton gradient. Because, there is a positive charge on the outside here, there is a negative charge on the inside. But, since we require the protons for the ATP to be produced, protons have to be pumped, where? To the inter membrane space. That is what we have here.

So, we have a P side and we have an N side. The N side is the negative side. Where is this N side? It is the matrix of the mitochondria. Where is this P side? It is the inter membrane space of the mitochondria. This is where a higher concentration of protons exist, but we require an even more amount of protons for ATP to be produced. So what you have to do is, protons have to be pumped, from this to that side. That is essentially what is happening. Now, because of this negative and positive side here, you have a membrane potential development.

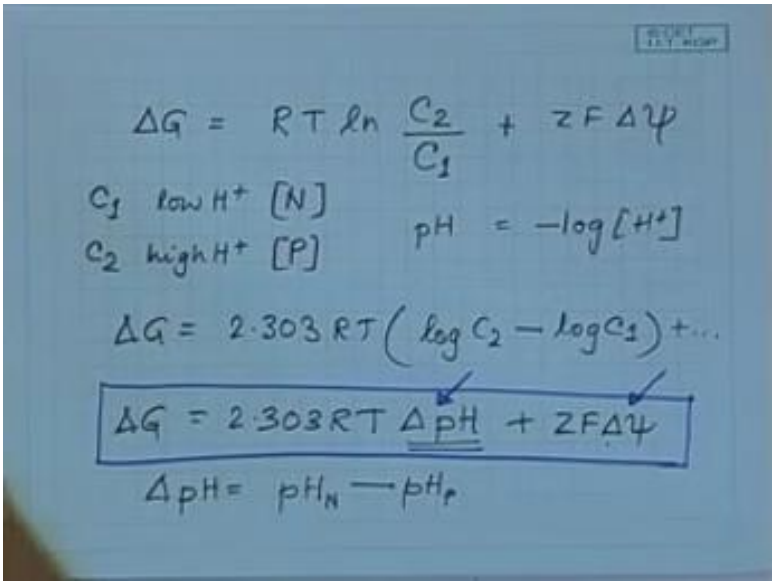
(Refer Slide Time: 43:21)



What is this membrane potential? We have a membrane potential developed, so, when an ion is transported from negative to positive delta side, which is the membrane potential is positive. Because you are going from a negative value to a positive value. So what is the delta side, it is a positive value. Now, when you have the matrix side, the matrix side is the N side, that has a low proton, a low hydrogen ion concentration, a low proton concentration, a negative electrical potential.

The inter membrane side, which is the C 2 concentration in this case, has a high proton concentration and has a positive electrical potential. So, when you are looking at the delta G values, you have an RTln, what is your product in this case? It is going to the inter membrane side, so it is C 2/ C 1. You have a ZF delta side, which is nothing, but your NFE. It is a potential. Now, when you consider the low H+ concentration and the high H+ concentration, you can link the logarithm of the hydrogen ion concentration with the PH.

(Refer Slide Time: 45:05)

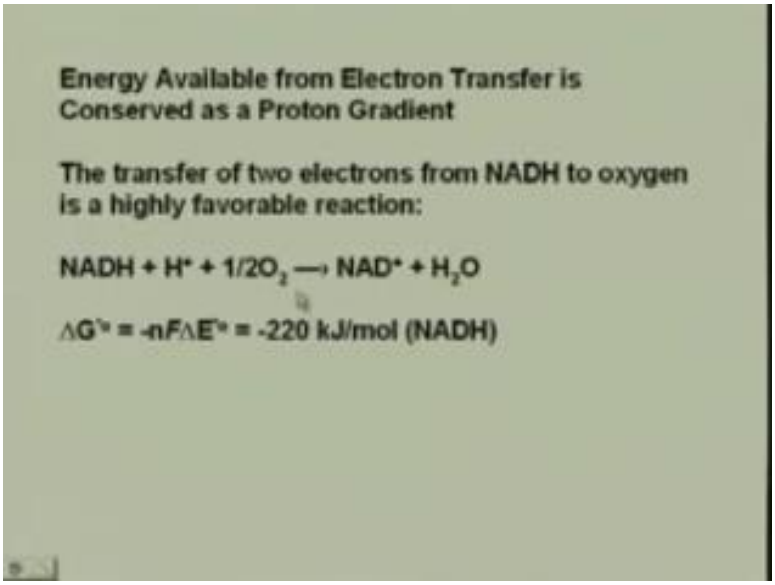


So, if we just work this out, I have my delta G. My delta G is RTln, C 2/C 1. Where is my C2? My C 1 is low H+. My C 2 is high H+. And you have to remember that the high H+ is in the inter membrane space and you are still pumping in H+ to that space. Why? Because, you have to make ATP. This is plus ZF delta side. Now, if you want to convert, what do we know? We know that the PH is equal to minus log of H+ concentration. We know this.

So, all we have to do is, relate this with the PH. So we can write this, 2.303 RT, it is going to be log of C 2 minus log of C 1, plus your other part. So, what do we have here then? It is going to be your 2.303 RT, a delta PH, where, what is this delta PH going to be equal to then? The PH of that is the N going to PH P side. What is this, where is the low H+? It is on the N side. Where is the high PH, this is on the, high H+, it is on the, high H+ means low PH.

So, we have this relation. This is your delta G. So, basically what you are doing is, you are using this relation, based on the delta PH. What is this delta PH? It is the difference of the hydrogen ion concentrations, between the matrix and the inter membrane space. This is the membrane potential. What membrane potential? It is the inner membrane potential. So, we have the energy associated with the proton gradient

(Refer Slide Time: 47:39)

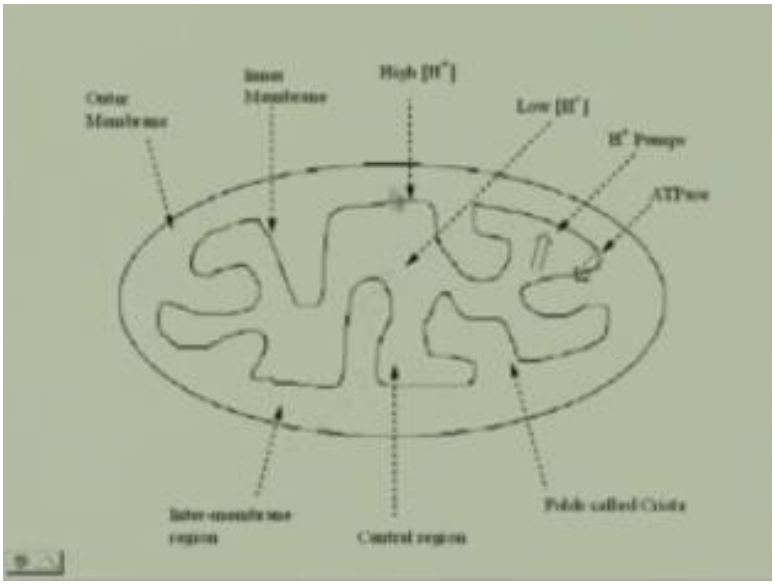


Now, when we look at the energy available from electron transfer, it is conserved as a proton gradient. And, what happens is, when we transfer this, when we have this reaction of NADH going to NAD plus, you recognize that if NAD plus is going to form NADH, there has to be a reaction that is going to get it back to NAD plus, so it can be reutilized. Just like you would have the enzyme. So similarly, as we are breaking down the ATP, we have to produce ATP.

And we will look at the production of ATP also. So, there are certain reactions, for example this reaction that will more than compensate, for the amount of the delta G zero prime that you need for this proton pump. You see, what a very large amount of energy this is, -220kJ/mol and this energy will be utilized for your proton pump to maintain the proton gradient. And why do we have to do that? We have this proton pump, we have the H+ to produce ATP. That is why we require this.

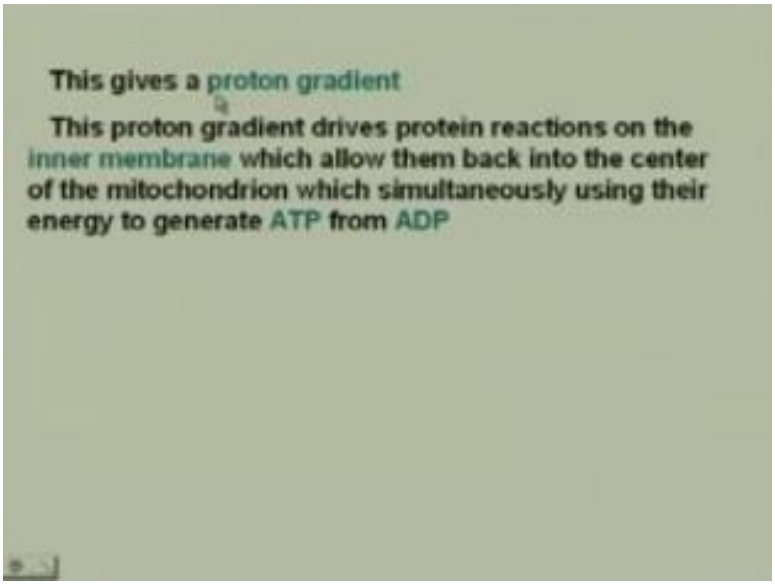
So, this is what the proton pump is. We have our specific requirement, where the proton have to be pumped from the negative side to the positive side, for the production of the ATP.

(Refer Slide Time: 49.00)



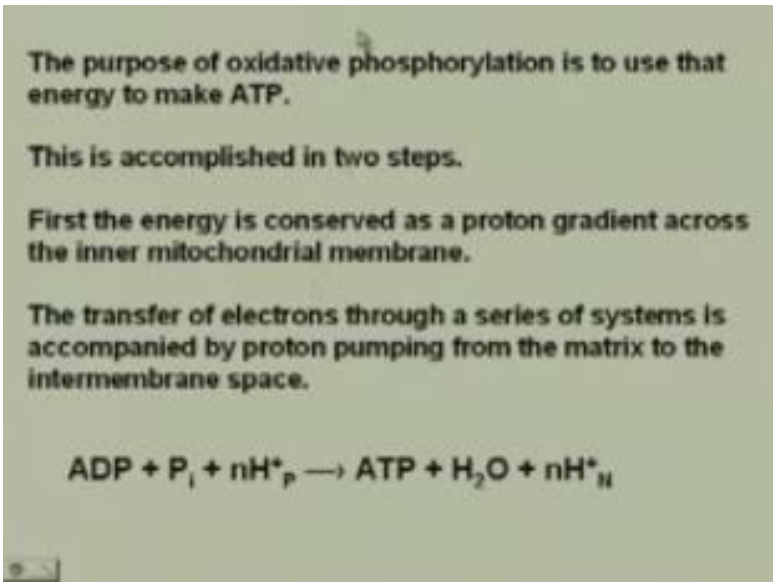
So, we have basically the picture of the mitochondria here. This is the outer membrane. And both of these are lipid bilayers. We have an outer membrane, we have an inner membrane. Within the inter membrane space is high H+ ion concentration. We have a low H+ here. The hydrogen+ is pumped from the lower H+ concentration to the high H+ concentration because, ATPase, there is a protein called F 0 F 1 ATPase, ATP synthase, that does nothing but synthesize ATP.

(Refer Slide Time: 49:49)



And the proton gradient, this proton gradient drives protein reactions on the inner membrane, which allow them back into the center of the mitochondria, which uses this to generate ATP from ADP. Because essentially what you want to do is, you want to produce ATP, which is why you are pumping all the protons, into the inter membrane space, for the production of ATP.

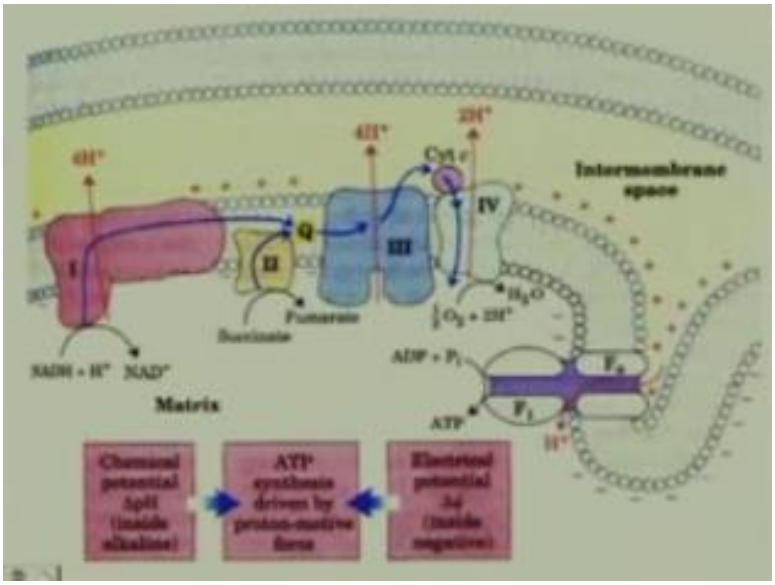
(Refer Slide Time: 50:18)



Now, this ADP, the purpose of the oxidative phosphorylation is to use the energy to make ATP that is accomplished in two steps. First, we have the proton gradient. Then, we have a transfer of electrons through a series of systems. We are not going into the details of the systems. But, this is the essential reaction that takes place, where n is above three. So it means that we have to pump in three protons, for the production of one ATP.

So, three protons have to go from where? We have to get for the ATP. We have to go from ADP + P i and H+ p, that is the positive end, to ATP + H 2 O in the negative. So, in the matrix side, the ATP is produced. That is why, why is it produced there? It is because all the reactions that are taking place are in the, they don't happen in the inter membrane space, all of the enzyme, enzymatic reactions occur in the matrix space.

(Refer Slide Time: 51:25)



Now, what we have, actually I will just show you this. This is where we have the inter membrane space, where we have, you see a large number of positives. We have a protein that is called the ATP synthase; have the details of which we will do in the next class, where we are looking at a series of reactions that are actually going to get us into the formation of ATP. So, what we learn today was how we can actually have, derive from vitamins, specific cofactors and coenzymes that are going to result in couple reactions.

We have the ATP, ATP, the hydrolysis of the ATP is going to give us enough energy to couple with another non spontaneous reaction to give us a spontaneous reaction. Like the example that I showed you, with hexokinase, we are going from glucose to glucose six phosphate, and we are getting the phosphate from the breakdown of the ATP. In the dehydrogenase or oxidase reaction, we are using NAD plus or FMN or FAD that are going to be couple with enzymes, such as dehydrogenases or oxidases.

Because, they have to lose, the compounds have to lose their hydrogens and these are going to be utilized all in the redox reactions, they are going to be taken up, so either you have to have a reduction or an oxidation. And based on what reaction you have, the enzyme is going to have as a cofactor, either NAD plus or FAD. So, what we will see in the next class is how this ATP is actually formed and then we will then go on to the metabolism of carbohydrates. Thank you.