Medicinal Chemistry
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Mechanisms in Biological Chemistry Part 1

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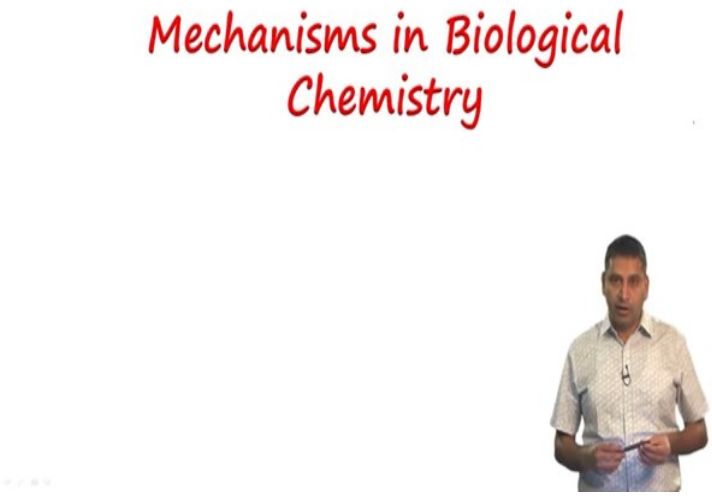

Welcome back. So in today's lecture we are going to look at some important mechanisms that happen in biological chemistry. So far we have covered a number of topics related to how a protein functions, how a receptor functions and so on. The next set of topics that we are going to be looking at is ADME which is absorption, distribution, metabolism and excretion. So before we get into those topics we will look at broadly some of the mechanisms that happen in biological chemistry and what we will do is to look more at arrow pushing mechanisms so I am sure that all of you have sort of being exposed to very good levels of organic chemistry, where you have been doing number of reaction mechanisms which involve arrow pushing.

So what we will cover today is the some of the major mechanisms that happens inside the cell, okay.

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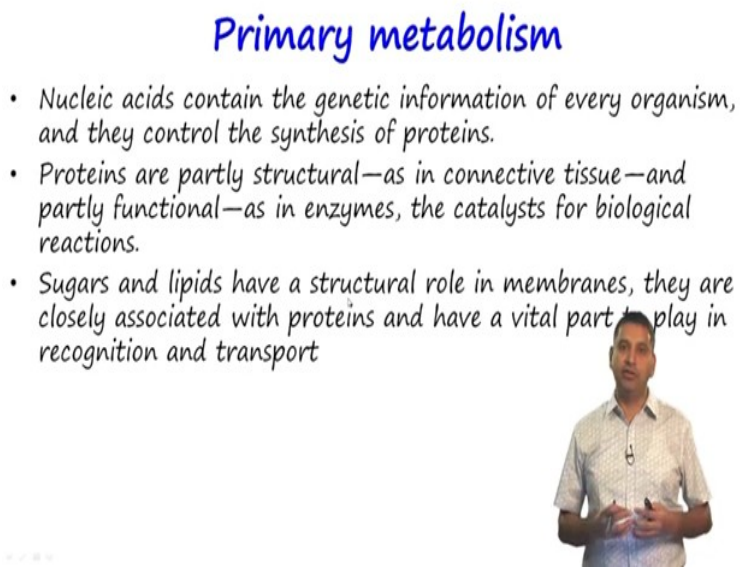

So to understand this basically what we are going to deal with is primary metabolism and again we will not go into great depth into primary metabolism but we will do couple of lectures of this. So as we have looked at you know previously in the course nucleic acids are the ones that contain the genetic information for every organism and they help with synthesis of proteins we have already looked at the scheme through which this happens.

Now proteins have diverse functions, so one of them include you know making connective tissue or you know an enzymes to carry out catalysis, they can be on the surface as receptors and we have already seen that sugars and lipids have a major structural role in membranes. So the lipid bilayer for example is important in making sure that you know the contents of the cell are not sent out in an unregulated manner, okay and of course sugars also have an important role in cell recognition so one cell can sort of interface or understand what is going on with another cell through the sugar based recognition motives.

So we have covered some aspects of these, but if you want to look at metabolism, metabolism is the process by which the you know the sugars are broken down for example into carbon dioxide and water and there are any process that happens inside the cell which involves breaking down and consumption of energy or generation of energy would be considered as metabolism.

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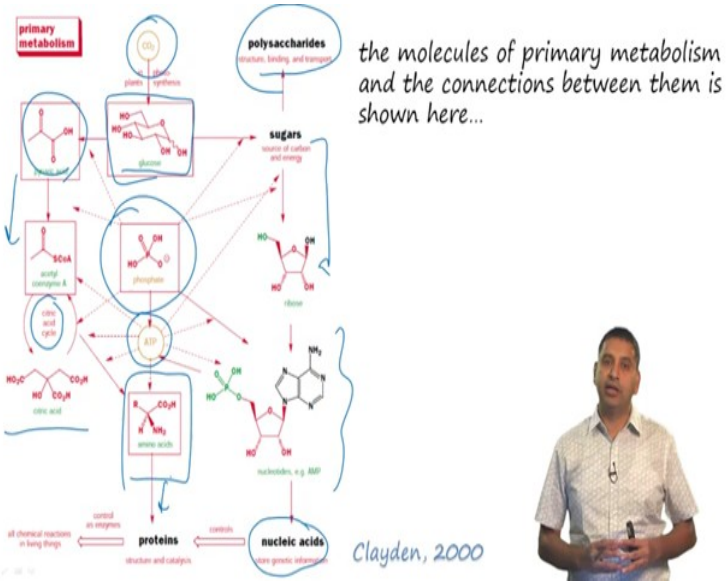

So this is a complicated sort of figure, so we will not go into too much of depth but there are number of important molecules here that we need to sort of look at. So the first one is basically carbon dioxide which is fixed by plants to produce glucose and as we all know glucose is the major source of energy for us and glucose can of course form polysaccharides which through you know through not just glucose but other sugars can form polymers and that forms polysaccharides.

It can also be broken down into pyruvic acid which then forms acetyl-CoA and acetyl-CoA as we know can enter the citric acid cycle to produce citric acid. Now acetyl-CoA is also important in the synthesis of amino acids and we should look at some of these aspects later in the course in the lecture and of course phosphate plays a very central role because it is important as a molecule for phosphorylation. We will look at how phosphate is going to be key player in enolate chemistry for example and it is also important in ATP and ATP is the major energy currency inside the cell and ATP is going to also help with phosphorylation of certain residues, okay.

And of course sugars are going to form ribose for example ribose is a sugar and ribose is a part of nucleic acids as shown here like for example AMP and so on and it comes full circle because nucleic acids is what we started with is going to control how proteins are synthesized and proteins are going to carry out a large number of reactions within the cell and of course amino acids are comprised proteins are comprised of amino acids which are again some of them are synthesized through the from acetyl-CoA.

So although this picture looks very complicated, it is highly interconnected and each of these arrows here indicates a number of enzymes that can do this process and these occur in a very highly coordinated and regulated manner inside a cell.

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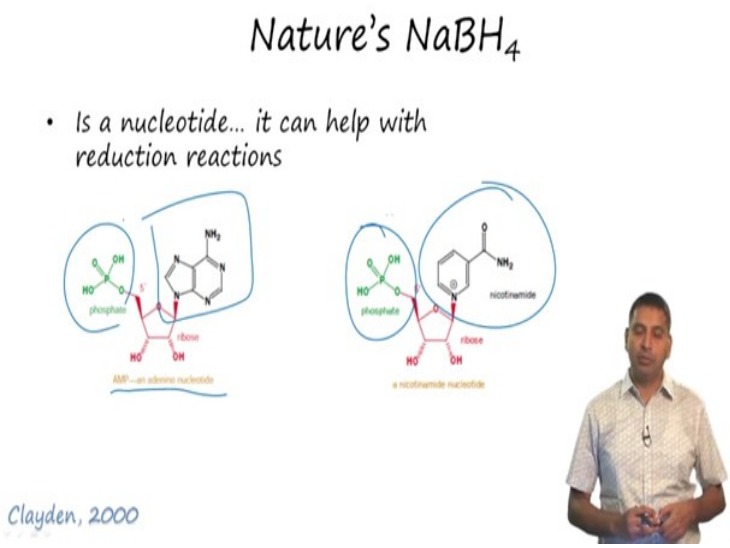

First let us look at the one of the important reactions inside a cell which is oxidation and reduction. So of course as you will recognize sodium borohydride is a very important reducing agent in organic chemistry, but there is no sodium borohydride inside a cell so we will have to you know we resort to other reducing agents and the nature's sodium borohydride is actually this molecule which is shown here which is basically nicotinamide and if you see the structure of nicotinamide or NAD it looks very much like the AMP which is adenosine mono phosphate and you can see the ribose sugar is identical and you also have a phosphate group here and the major difference here is in the nucleobase. So if you see here this nicotinamide has a positive charge on the pyridine ring.

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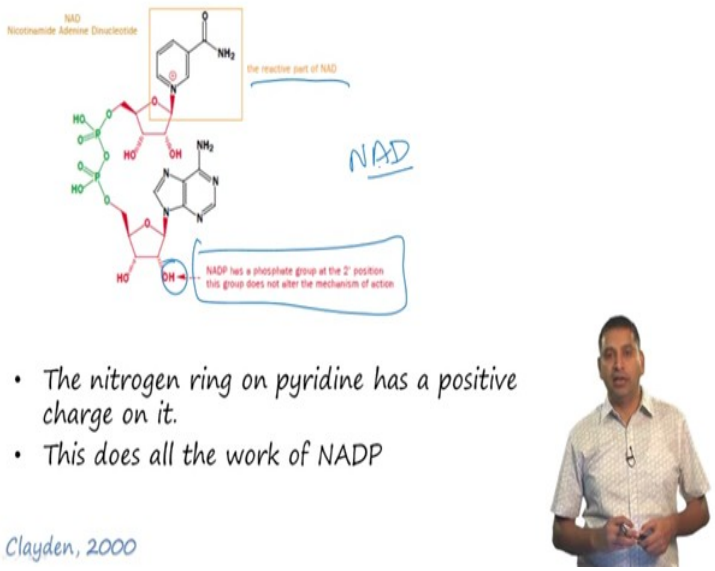

So that is the business end of the molecule and this is what does the reduction and oxidation in the cell. NADP has a phosphate group which is attached at this 2 prime position, otherwise it is just called NAD and so that is the distinction between NAD and NADP and in certain cases NADP is used and in certain cases NAD is used. So as I mentioned earlier the nitrogen on the pyridine has a positive charge and we will come back to this very shortly.

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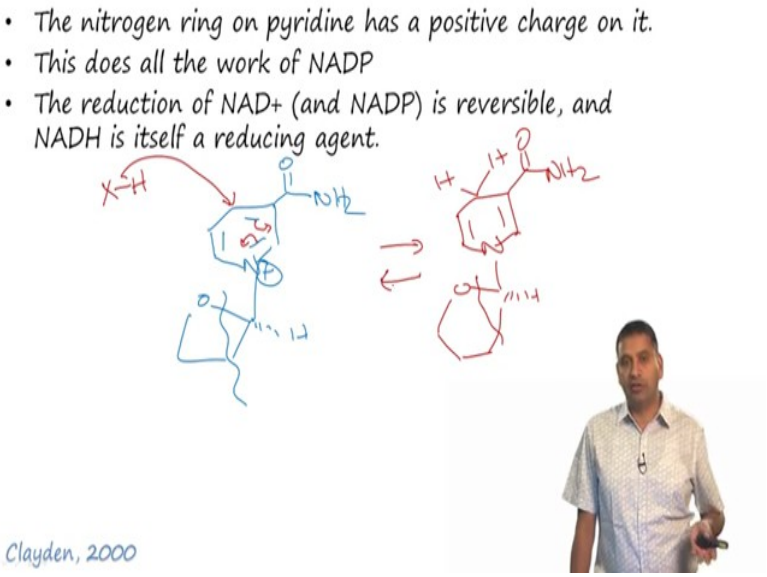

So this positive charge is what is going to help with doing a lot of work. So now let us just draw out the business end of the molecule which is the pyridinium ion so which has N and you have a amide over here C double bond O NH_2 and there is a positive charge and there is a

hydrogen and this is your sugar, okay so we are not going to draw the rest of the molecule. So here is the positive charge on nitrogen.

So what this does is that it makes the centre which is *para* to it quite susceptible to attack. So what happens is that you can imagine that there would be some source of hydride which can come in and donate it to this molecule and then you can push arrows in the following manner to give you the product which is basically the this molecule here with the rest of the sugar essentially remaining intact, okay.

So this is how NAD functions and you have now a molecule which can accept hydride and you can draw exactly the reverse reaction wherein it is actually going to donate hydride and in which case it is going to be act as reducing agent. So this is a highly reversible process and it helps with regulating or carrying out a number of oxidation and reduction reactions inside the cell.

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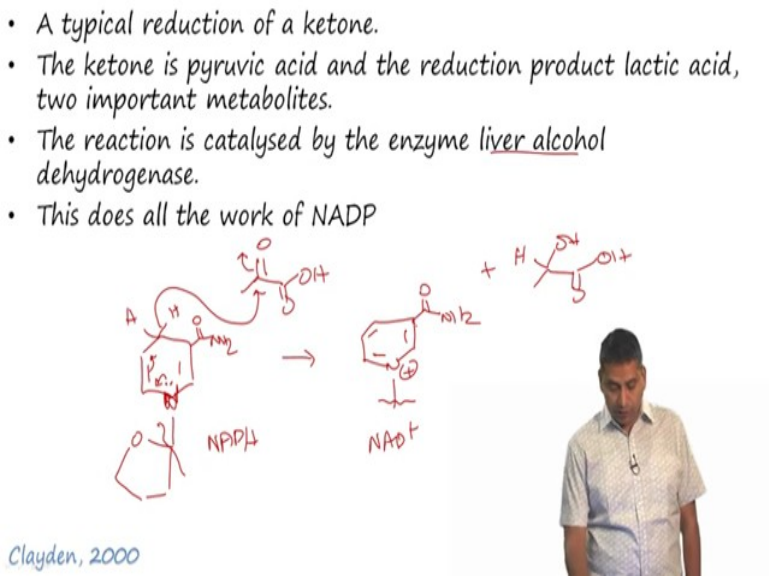

So let us look at a typical reduction of a ketone and the ketone that we are going to look at is let us draw an NADP once again here is the reduced form that is 2 hydrogens over here, nitrogen is here, here is the sugar and the bottom part of the molecule is important but we are not going to consider it for the mechanism and if you imagine that the ketone is basically from pyruvic acid.

Now you can push electrons like this going in here, going in here and this hydride now attacks the carbonyl and reduces the ketone to form a alcohol. So the product in this case would be the pyridinum ion so there is a double bond here, double bond here, this double

bond remains intact and of course the NH_2 is still here $CONH_2$ ad you now have a positive charge on the pyridinum ion and pyruvic acid has now become so you have the reduced form of pyruvic acid as shown here and this is going to be the product, okay.

So here is NAD plus and this is NADH and so this is called this is the nature's reducing agent that we need to be able to understand and the reaction that we are looking at here is the liver alcohol dehydrogenase enzyme, okay.

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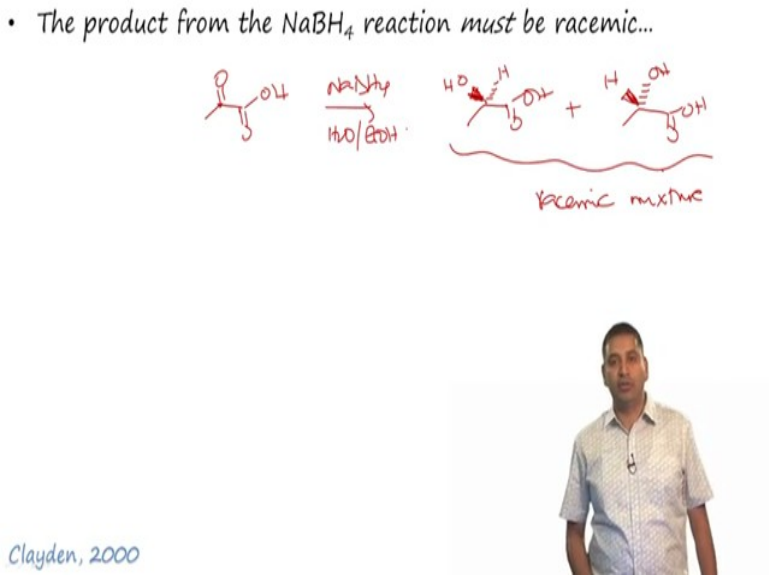

Now the product from $NaBH_4$ reduction so if I just take regular ketone for example let us say we take pyruvic acid and react this with $NaBH_4$, now hydride can attack from the top phase or the bottom phase and you will end up with let say it attacks from the bottom phase you have hydride over here and if it attacks from the top phase you have hydride over here, OH here so you will end up with a racemic mixture, right.

So this is what you would expect with regular sodium borohydride, right and you can imagine that this can be you can have water and ethanol as potential solvents in this reaction, okay.

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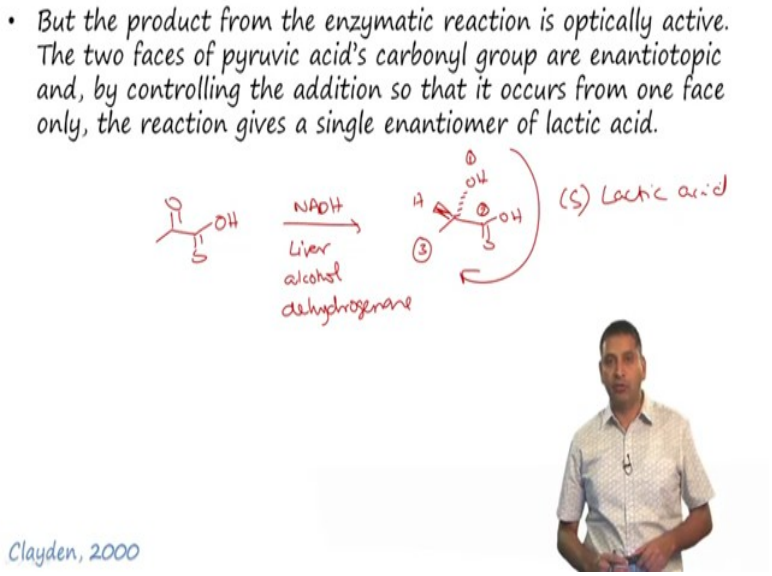

But when you do this reaction in an enzymatic manner the product is actually optically active. So the two phases of pyruvic acid which are enantiotopic seem to be reacting in a way in which you get only one isomer, okay. So when you start with pyruvic acid C double bond O OH when you reacted with NADH and liver alcohol dehydrogenase, okay so you end up with a molecule where the OH is over here and COOH is here and so hydride essentially transfers in the top.

So, now let us assign the stereochemistry for this molecule so the priority here is 1, the priority is 2, the priority is 3 and so this is clockwise but hydrogen is facing us so this becomes S, okay. So when you react pyruvic acid with NADH in the presence of an enzyme such as liver alcohol dehydrogenase you end up exclusively with S lactic acid, okay. So this is one of the very interesting aspects of how nature carries out its reduction reactions in a very enantiospecific manner.

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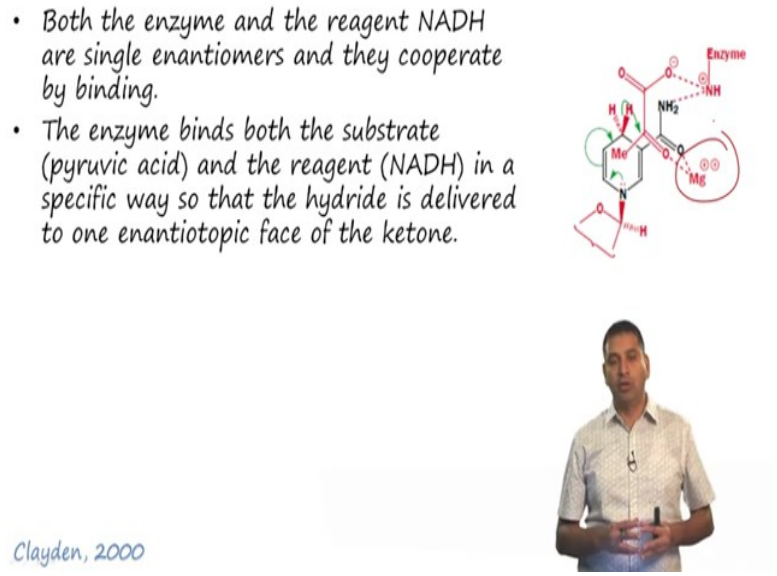

So in order to understand this we can very well appreciate that one of the enantiotopic phases is going to be preferred over the other. So when we then look at the binding site and look at the various residues present in the binding site model has been proposed where pyruvic acid is actually held by weak interactions with the amine one of the amine residues of the enzyme and you have this magnesium ion chelating with the two carbonyls which are going to play a key role in this process.

One of the carbonyls comes from the amide which is present in NADH and then there is a transfer of hydride from the phase which is from the bottom phase and you end up with this product, okay. So there is a some sort of a cooperativity that is achieved in the active site of the enzyme and hydride is delivered only from a single phase.

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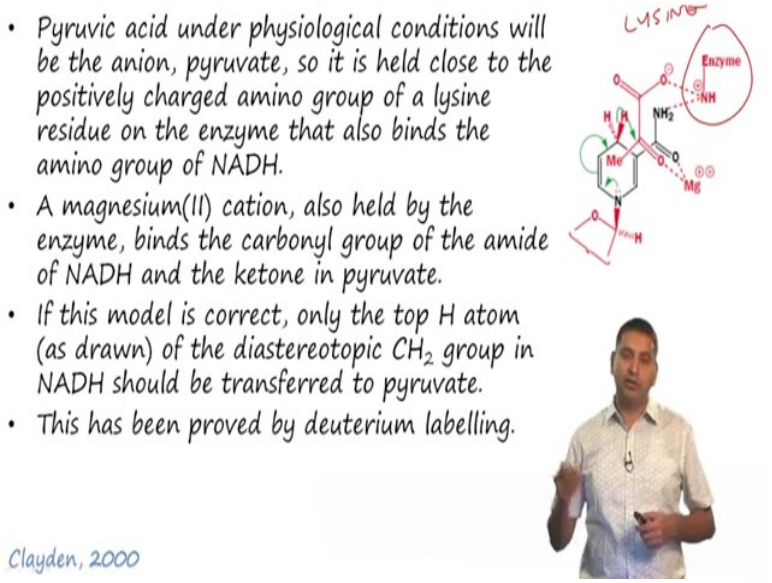

Now pyruvic acid under physiological conditions will be an anion and so pyruvate is held closely to the positively charged amino group, okay and so as I mentioned earlier this amino group is from lysine and this lysine can then help with binding to the carboxylate and of course the magnesium cation is also plays a key role in this process. If this model is correct only the top hydrogen of the diastereotopic phase in NADH should be transferred to the pyruvate.

So if you then specifically label one of the hydrogens converted to deuterium, we would be able to figure out whether this is true or not and this has indeed been done and it has been shown that the top hydrogen is the one that is reacting with pyruvate.

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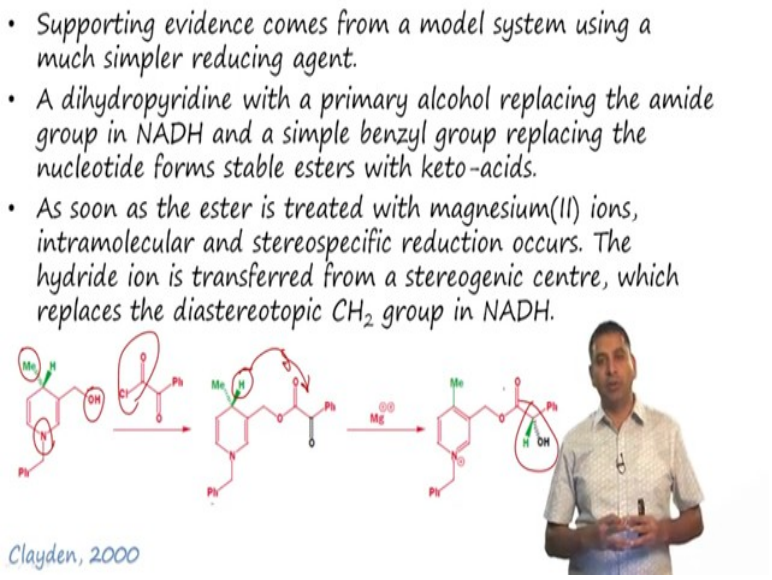

So supporting evidence comes from a model system which uses a much simpler reducing agent. So what was done here is that a dihydropyridine with a primary alcohol was first synthesized. So notice here that this methyl group has been installed in a very stereo selective manner so that you can distinguish or discriminate between the two phases, then what we do is we reacted with this acid chloride to give you the corresponding ester.

Now when you add magnesium ions this hydride is actually transferred and reacts with the ketone. So once the ketone is once it reacts you get this product which is shown here. So as soon as the ester is treated with magnesium ions there is an intramolecular and highly stereo specific reduction that occurs and the hydride is transferred from the stereo geniccentre, which replaces the diastereotopic CH_2 in NADH.

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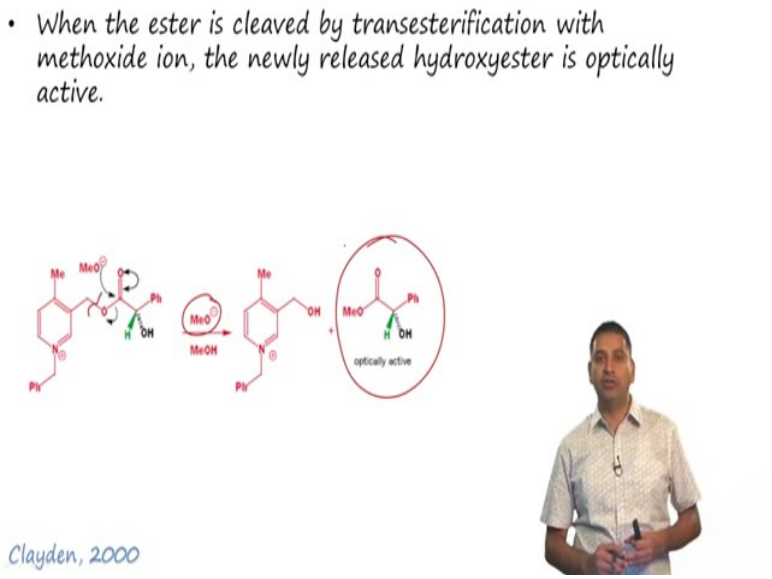

So when the ester is then cleaved by transesterification with methoxide ion, so you add methoxide and cleave this ester bond and what you get is optically active molecule here which is a single enantiomer.

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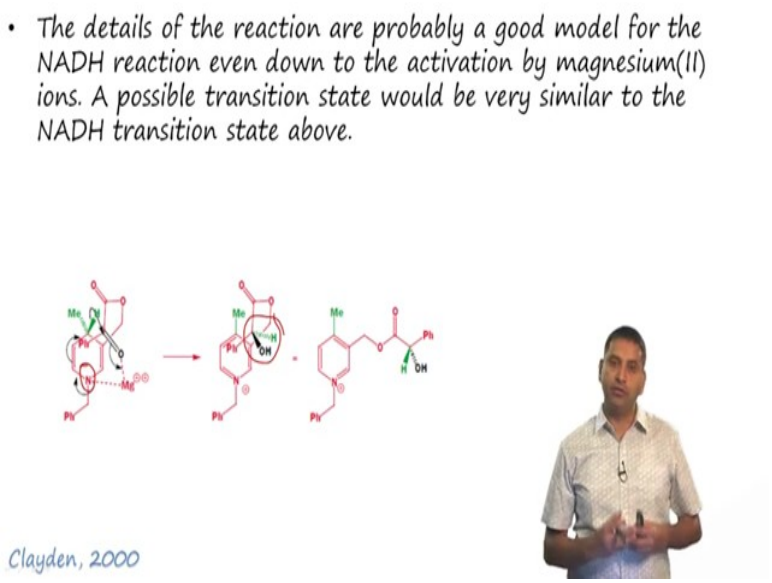

So what we can use this model system for is to propose sort of a reaction mechanism. So what possibly happens is that you may have a transition state which should be very similar to the NADH transition state as described previously and what may happen is that this molecule sort of comes in and it turns around and accesses the hydride and once you add magnesium ion, magnesium can coordinate with the pyridine with the nitrogen over here and the carbonyl and this places the molecule or sort of locks the molecule in a particular state conformation through which the hydride can be transferred and then once the hydride is transferred it happens in a very selective manner and gives you the product as shown here. So such model systems are very useful to understand mechanisms of these kinds of reactions.

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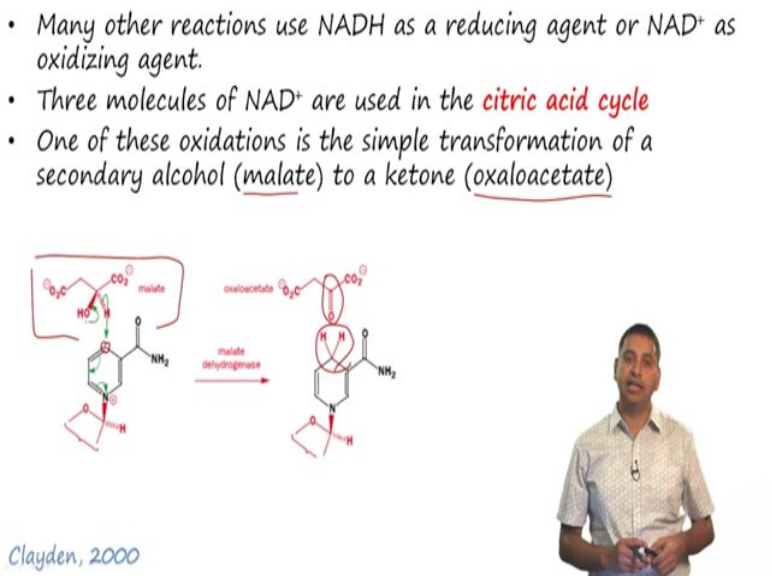

Many other reactions use NADH as a reducing agent or they use NAD plus as an oxidizing agent. So for example three molecules of NAD plus are used in the citric acid cycle and one of these oxidations is the simple transformation of a secondary alcohol which is malate to a ketone which is oxaloacetate. Now let us look at this reaction, so here is malate which is shown here and here is oxaloacetate which has the ketone as shown here.

So when NAD plus associates itself with malate then a reaction can occur wherein the hydride is transferred to this carbon as shown here and then from this carbon you can then push electrons wherein the nitrogen the positively charged nitrogen becomes electron sink and you end up with two hydrogens here and the NADH is formed.

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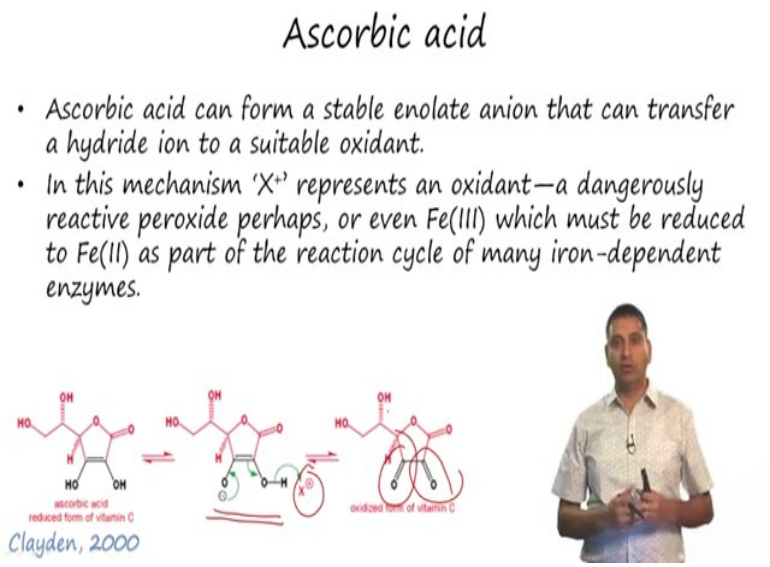

Now there are other reducing agents such as ascorbic acid, an ascorbic acid can actually form a stable enolate that can transfer a hydride to a suitable oxidant. So in this mechanism X plus which is shown here represents an oxygen and typically you know it may be a reactive peroxide or even Fe (III) which must be reduced Fe (II) as a part of the reaction cycle of many ion dependent enzymes.

So ascorbic acid which is nothing but reduced form of vitamin C which can then which can exist in this equilibrium as shown here and produce this kind of an intermediate and this is actually the enolate and the enolate can rearrange and give you the ketone as shown here and once it is transferred the electrons to the X plus then the X plus undergoes reduction.