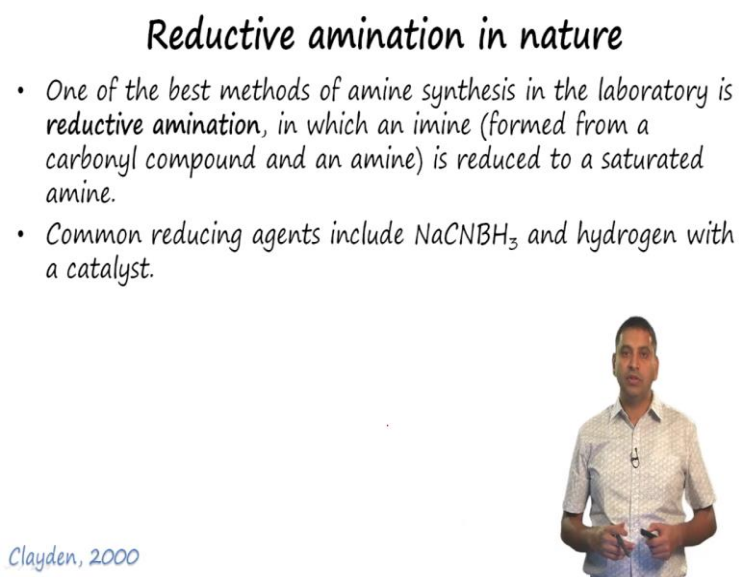
Medicinal Chemistry
Professor Dr. Harinath Chakrapani
Department of Chemistry
Indian Institute of Science Education and Research, Pune
Lecture No 30
Mechanisms in Biological Chemistry Part II

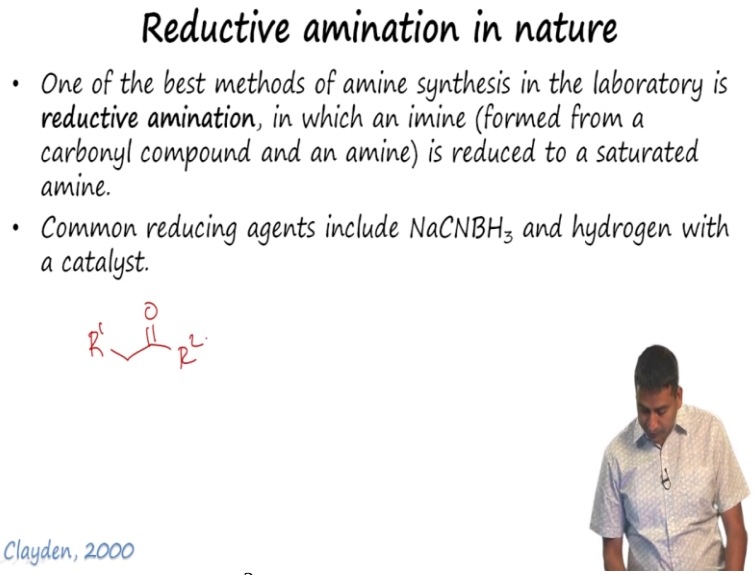
(Refer Slide Time: 00:16)



There is of course reductive amination is a very important reaction that we carry out in the lab and it also happens inside the cell. And as you can very well imagine that we do not have sodium cyanoborohydride inside the cell to carry out the reactions and so one must resort to other forms to do this.

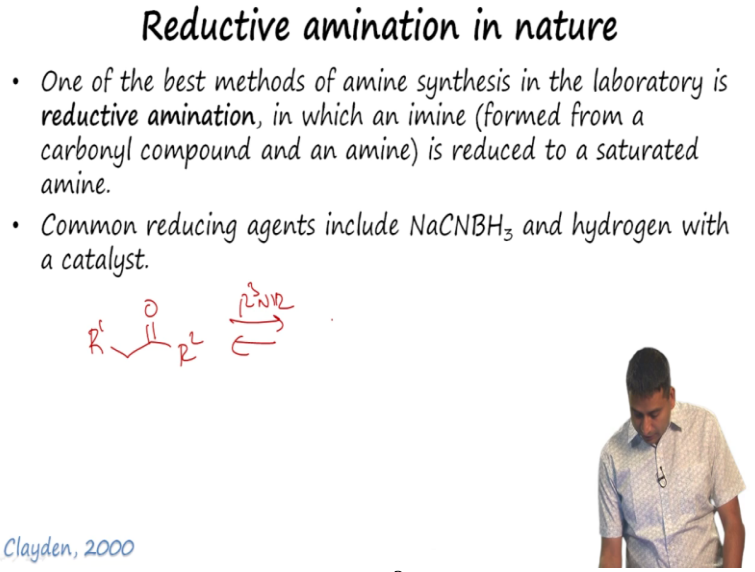
Let us look at the reductive amination as the possibility inside the, in the lab. So what you would start out is with the ketone such as this

(Refer Slide Time: 00:47)



which then, we can react this with R^3NH_2

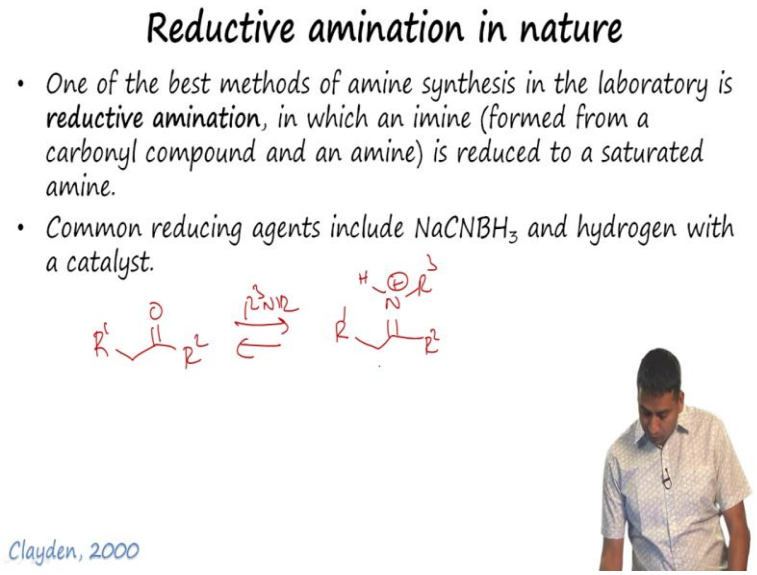
(Refer Slide Time: 00:53)



and this is going to give you an imine such as R^3, H and there is a positive charge. R^2 remains intact.

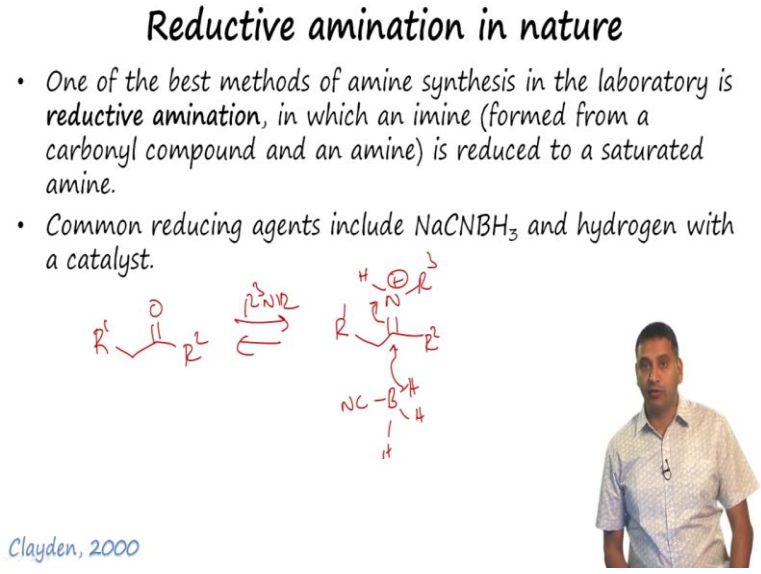
Now

(Refer Slide Time: 01:04)



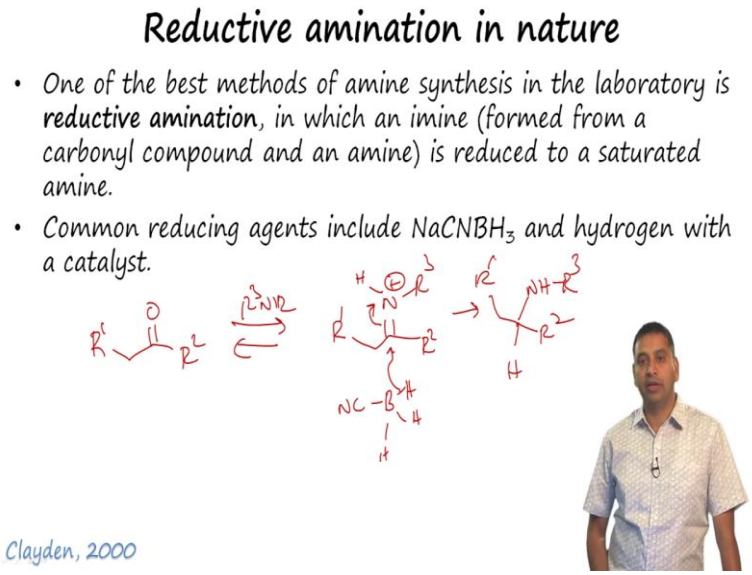
when we have sodium cyanoborohydride, right you can think of this

(Refer Slide Time: 01:14)



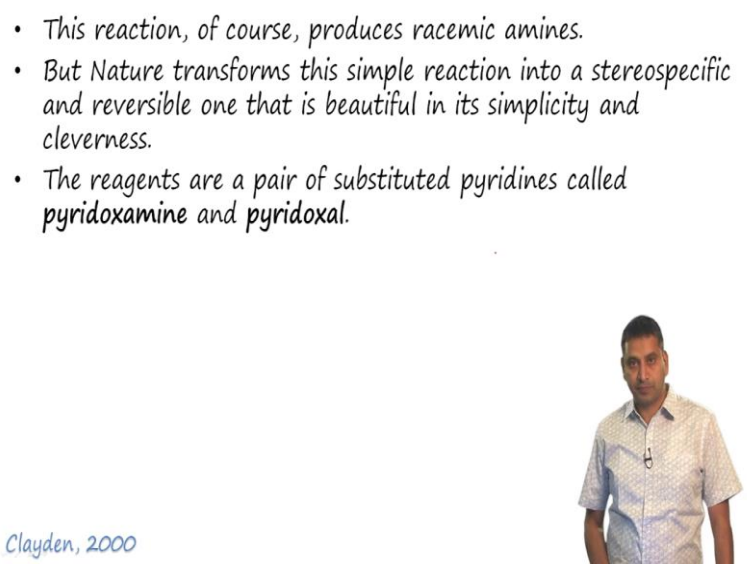
hydride transfer here, to give you, to use the nitrogen as the positively charged nitrogen as the electron sink and you end with NHR^3, R^2, H and R^1.

(Refer Slide Time: 01:33)



Ok. So this is a reaction that we can imagine very well can happen inside the lab. And now let us look at the biologically equivalent of this reaction,

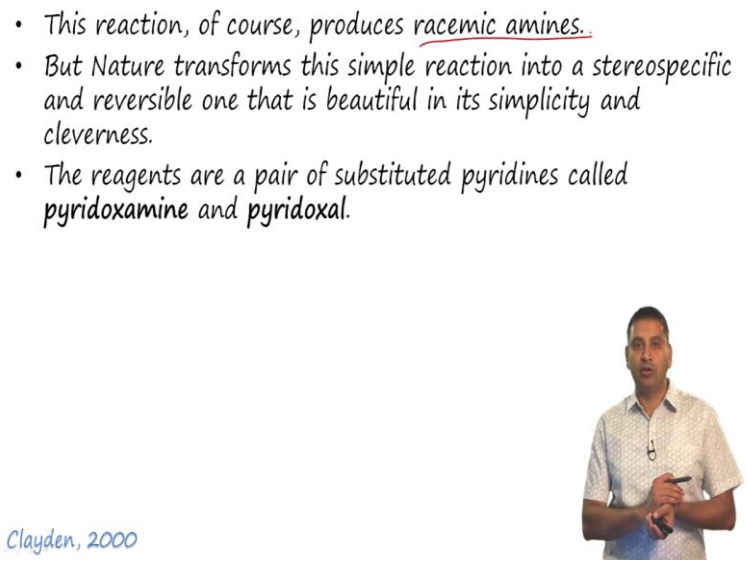
(Refer Slide Time: 01:42)



Ok.

And as you would predict it is going to, that there are two phases of the, of the imine that can undergo reduction and it is equally likely to, that either of these phases is accessed and so you end up with racemic mixture of amines.

(Refer Slide Time:: 01:58)

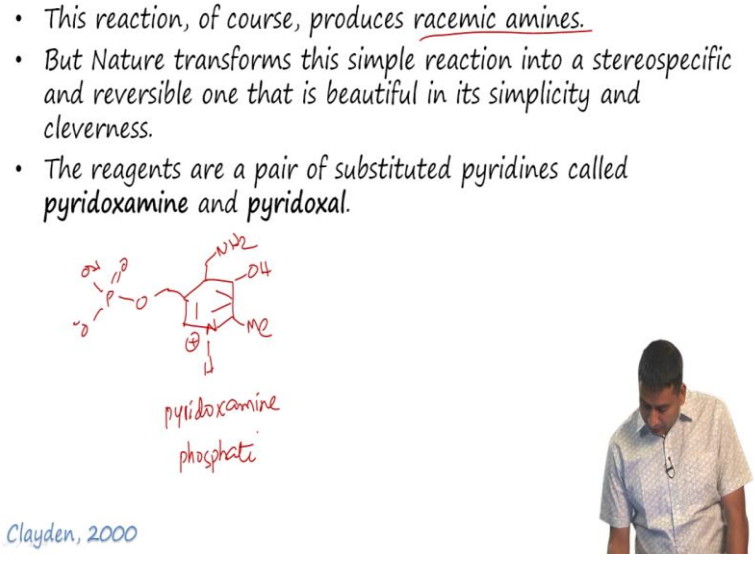


Ok.

But nature transforms this simple reaction into a stereospecific and reversible one that is not just beautiful for its simplicity but also for its cleverness. The reagents are actually a part, pair of substituted pyridines called as pyridoxamine and pyridoxal, Ok.

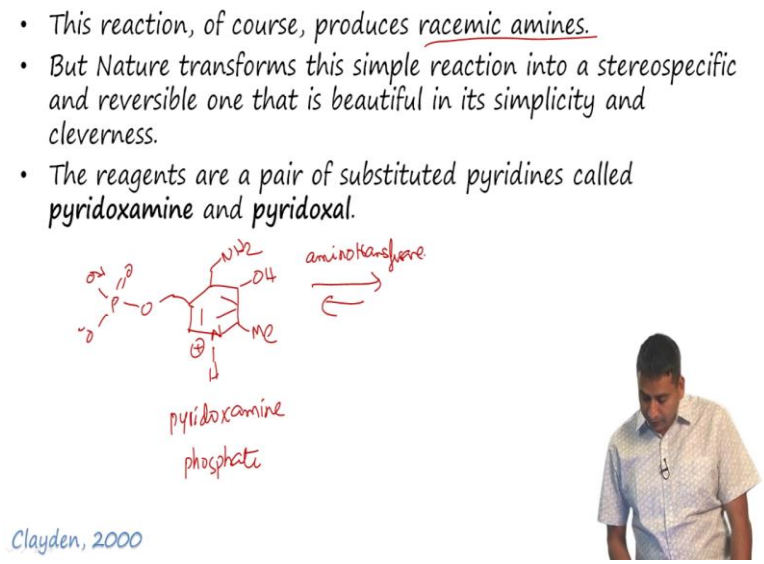
So we look at this reaction, reagents now. So this NH_2 is over here and this is giving you, you have phosphorylated P double bond O, OH, O minus. So this is pyridoxamine phosphate.

(Refer Slide Time: 02:56)



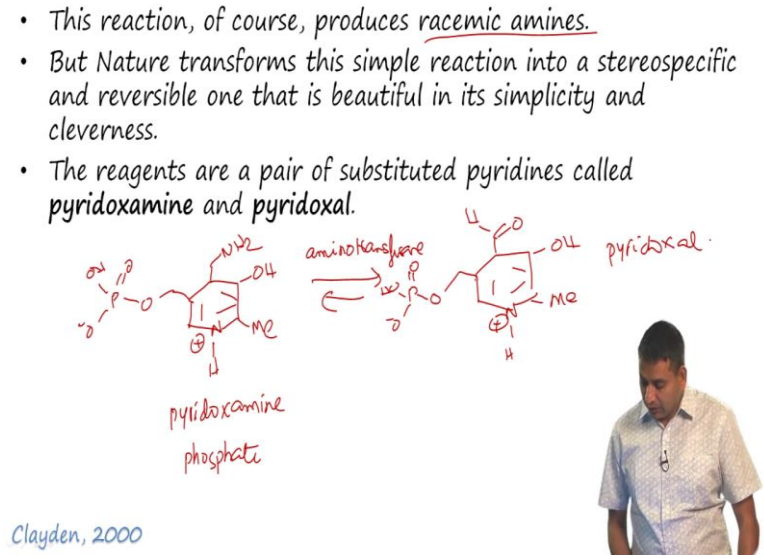
This can be transformed to the corresponding aldehyde through the enzyme called as aminotransferase,

(Refer Slide Time: 03:09)



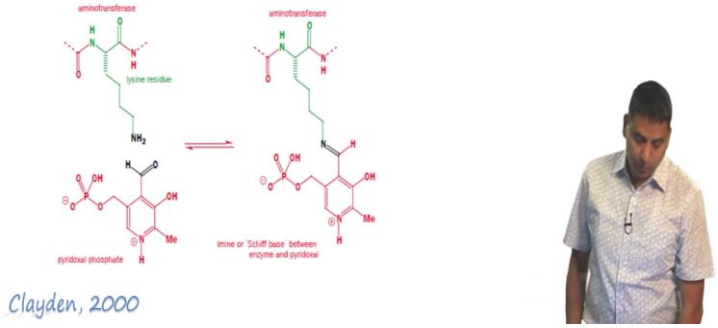
right and it is going to give you the corresponding aldehyde. And this aldehyde is called as pyridoxal phosphate. Rest of the molecule remains the same. Pyridine is still.

(Refer Slide Time: 03:40)



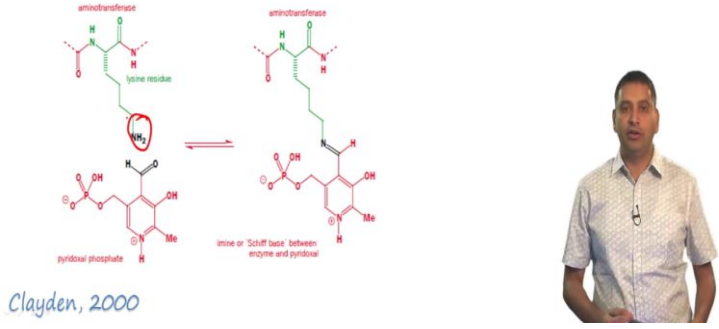
so the, this is the reaction we are going to consider now.

(Refer Slide Time: 03:48)



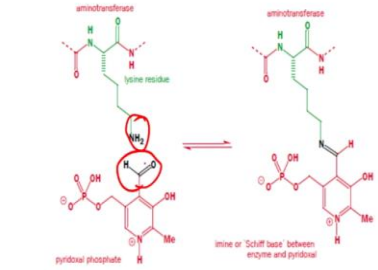
So, the way in which this reaction happens is that you, you would use, you would use a

(Refer Slide Time: 03:55)



lysine residue from the enzyme aminotransferase which is going to react with this aldehyde

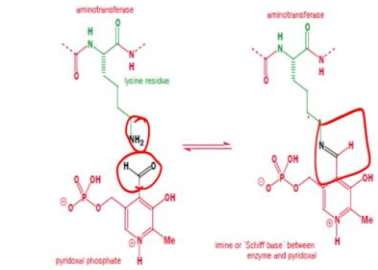
(Refer Slide Time: 04:01)



Clayden, 2000

to produce an imine, Ok.

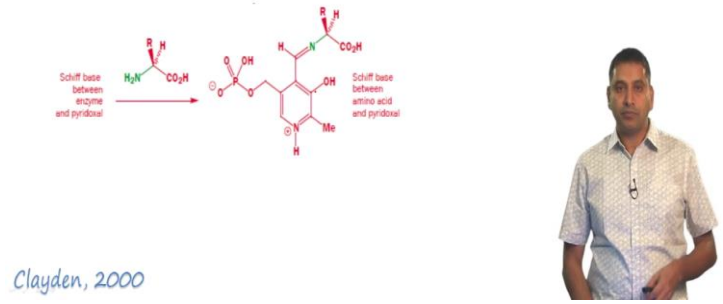
(Refer Slide Time: 04:03)



Clayden, 2000

So this imine is actually what is the Schiff base equivalent which can then undergo reduction to give you the corresponding amine.

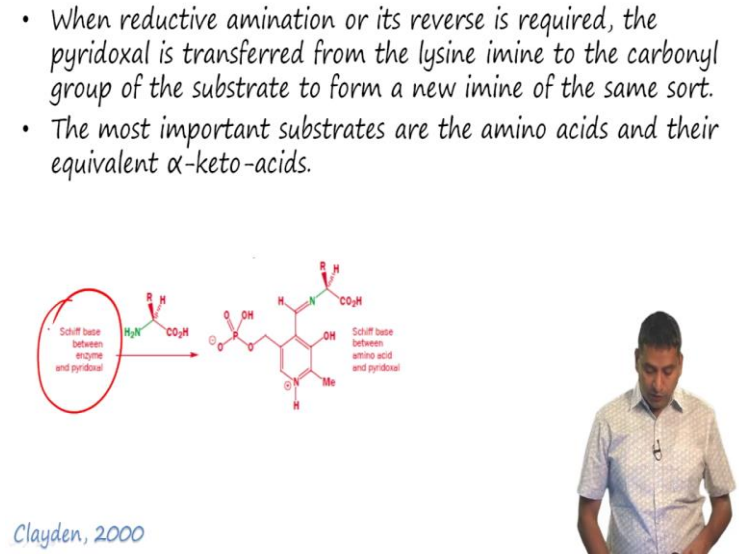
(Refer Slide Time: 04:12)



So when reductive amination or its reverse is required, the pyridoxal is transferred from the lysine imine to the carbonyl group of the substrate to form a new imine of the same sort. So the most important substrates are the amino acids and their equivalent alpha-keto-acids.

So here we need to consider is that

(Refer Slide Time: 04:34)

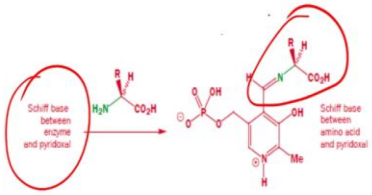


once you have the schiff base then, or the formation of the Schiff base is going to be the key step in this process.

So once the imine is produced, now

(Refer Slide Time: 04:44)

- When reductive amination or its reverse is required, the pyridoxal is transferred from the lysine imine to the carbonyl group of the substrate to form a new imine of the same sort.
- The most important substrates are the amino acids and their equivalent α-keto-acids.

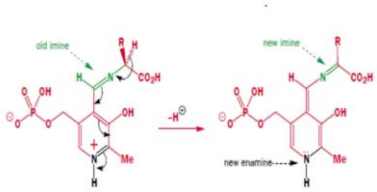


Clayden, 2000

this is going to

(Refer Slide Time: 04:46)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

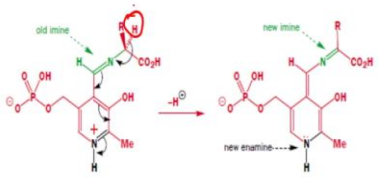


Clayden, 2000

then consider, then it is going to react further and give you the corresponding amine. So by using the protonated nitrogen of the pyridine as the electron sink, the alpha proton of the amino acid which is shown here,

(Refer Slide Time: 05:03)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.



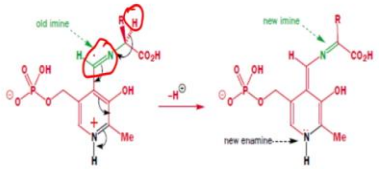
Clayden, 2000

right then reacts, is then removed to form a new imine at the top of the molecule and the enamine in the pyridine ring.

So let us look through this reaction. So this is the old imine that we started with.

(Refer Slide Time: 05:17)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

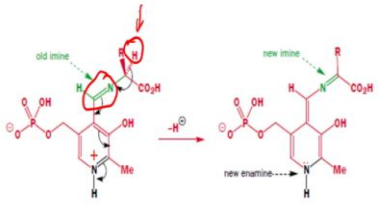


Clayden, 2000

And this hydrogen is then,

(Refer Slide Time: 05:20)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

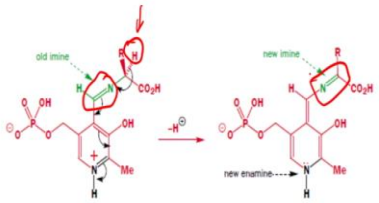


Clayden, 2000

is picked up and you form a new imine over here.

(Refer Slide Time: 05:24)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

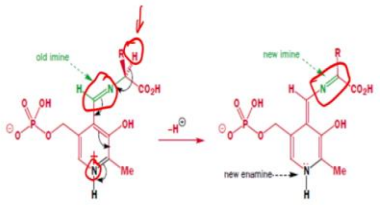


Clayden, 2000

And since this pyridine has a, is the

(Refer Slide Time: 05:27)

- By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

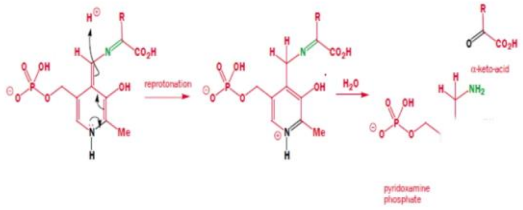


Clayden, 2000

electron sink you end up with a new enamine as shown here, Ok.

(Refer Slide Time: 05:34)

- Now the electrons can return through the pyridine ring and pick up a proton at the top of the molecule.
- The proton can be picked up where it came from, but more fruitfully it can be picked up at the carbon atom on the other side of the nitrogen.
- Hydrolysis of this imine releases pyridoxamine and the keto-acid.
- All the natural amino acids are in equilibrium with their equivalent α-keto-acids by this mechanism, catalysed by an aminotransferase.

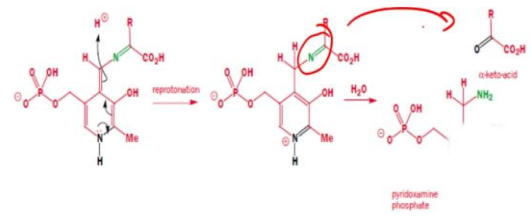


Clayden, 2000

And now the electrons can return through the pyridine ring and pick up a proton at the top of the molecule. The proton can be picked up where it came from but more fruitfully it can be picked up at the carbon atom on the other side of the nitrogen. So hydrolysis of the imine that is formed then gives

(Refer Slide Time: 05:52)

- Now the electrons can return through the pyridine ring and pick up a proton at the top of the molecule.
- The proton can be picked up where it came from, but more fruitfully it can be picked up at the carbon atom on the other side of the nitrogen.
- Hydrolysis of this imine releases pyridoxamine and the keto-acid.
- All the natural amino acids are in equilibrium with their equivalent α-keto-acids by this mechanism, catalysed by an aminotransferase.



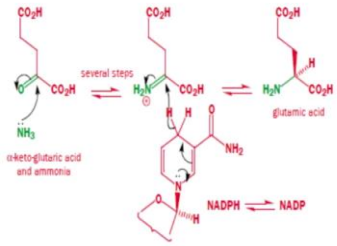
Clayden, 2000

you the alpha-keto-acid as shown here, Ok.

We will not go into the details of the hydrolysis mechanism of imines. I shall assume that you guys are familiar with it. So all natural amino acids are essentially in equilibrium with their equivalent keto acids by this mechanism. And this is catalyzed by aminotransferase.

(Refer Slide Time: 06:14)

- Reversing this reaction makes an amino acid stereospecifically out of an α-keto-acid.
- In fact, a complete cycle is usually set up whereby one amino acid is converted to the equivalent α-keto-acid while another α-keto-acid is converted into its equivalent amino acid.
- This is true transamination.

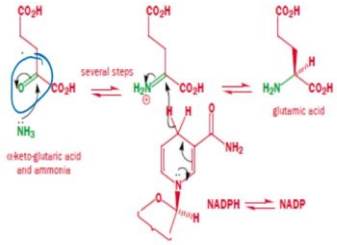


Clayden, 2000

Now reversing this reaction makes an amino acid stereospecifically out of an alpha-keto-acid, Ok. So what happens is that you can imagine or you can think, consider that ammonia can react with this alpha-keto-acid. In this case alpha-keto-glutaric-acid

(Refer Slide Time: 06:31)

- Reversing this reaction makes an amino acid stereospecifically out of an α-keto-acid.
- In fact, a complete cycle is usually set up whereby one amino acid is converted to the equivalent α-keto-acid while another α-keto-acid is converted into its equivalent amino acid.
- This is true transamination.

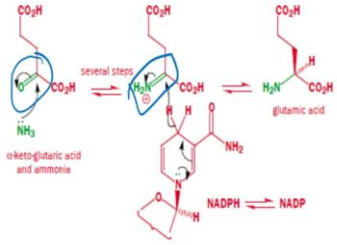


Clayden, 2000

and it will form this kind of an imine, right?

(Refer Slide Time: 06:34)

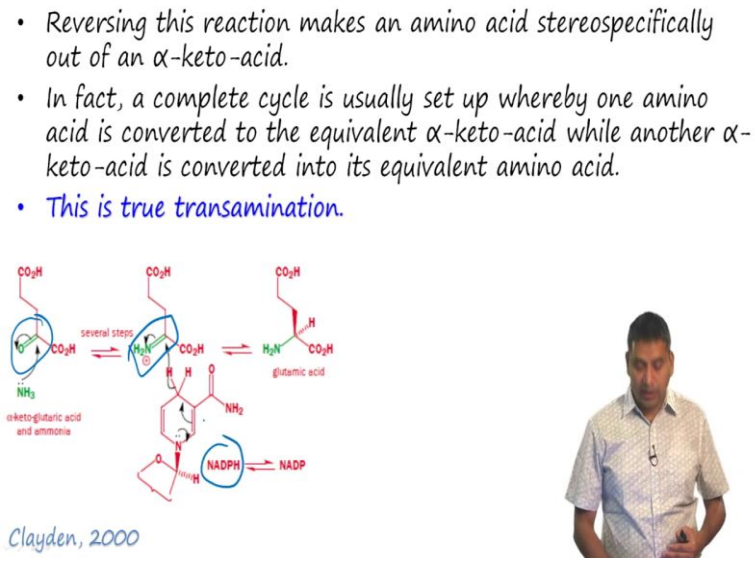
- Reversing this reaction makes an amino acid stereospecifically out of an α-keto-acid.
- In fact, a complete cycle is usually set up whereby one amino acid is converted to the equivalent α-keto-acid while another α-keto-acid is converted into its equivalent amino acid.
- This is true transamination.



Clayden, 2000

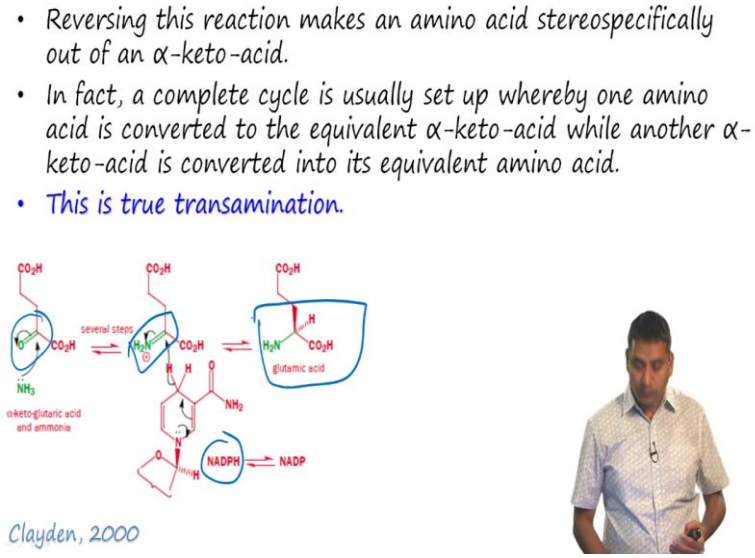
And this happens through several steps. Now reduction of this imine by NADPH as we have seen earlier can

(Refer Slide Time: 06:43)



occur. And this is going to give you glutamic acid as shown here,

(Refer Slide Time: 06:49)



Ok.

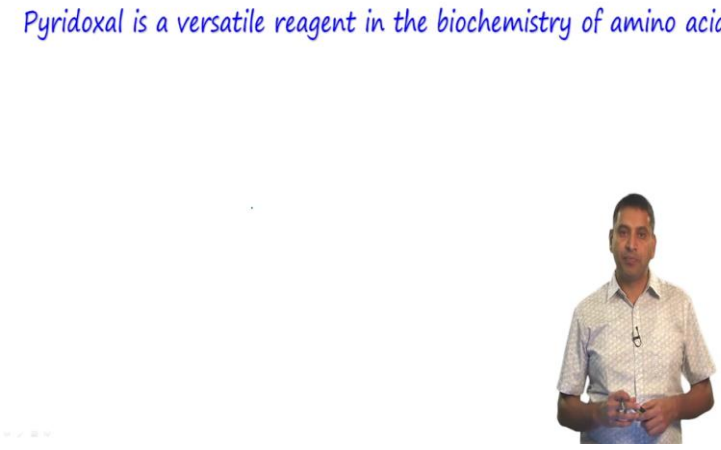
So therefore this process which is called transamination starts when you start with alpha-keto-carboxylic-acid. So we have looked at, in this, essentially in this process we have looked at a way in which an alpha-keto-acid can be produced from an amino acid.

And we have also looked at the reverse reaction where an alpha-keto-acid can be converted to the corresponding amino acid in a very stereospecific manner. So amino acids frequently get used up, for example in making proteins. So in order for life to keep going ammonia must be brought in from somewhere. So the key amino acid in this link is actually glutamic acid as we have seen below.

So therefore a true reductive amination using NADPH in ammonia builds glutamic acid from alpha-keto-glutaric-acid. The other amino acids can now be made from glutamic acid by transamination. We have already looked at these steps in the previous slides.

At the end of their useful life, they are transaminated back to glutamic acid which in mammals at least given its nitrogen to urea for excretion,

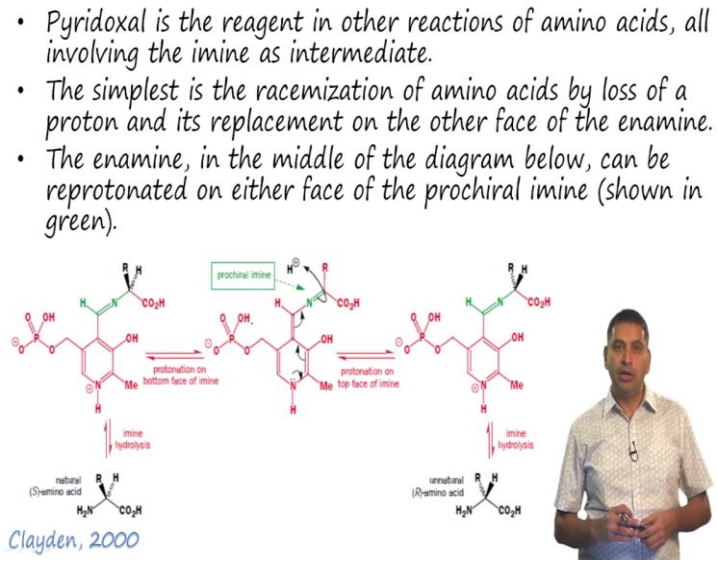
(Refer Slide Time: 08:03)



Ok.

So therefore pyridoxal is a versatile reagent in biochemistry of amino acids.

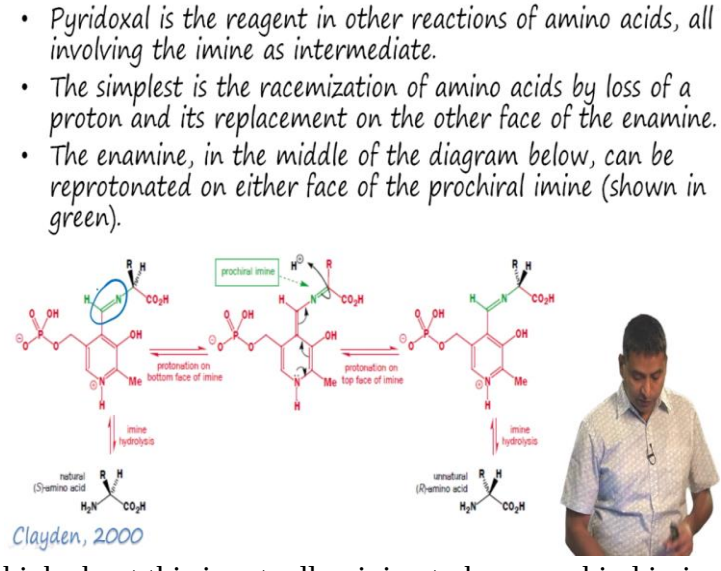
(Refer Slide Time: 08:09)



Pyridoxal is the reagent in other reactions of amino acids as well and they all involve imine as the intermediate. The simplest is the racemization of the amino acids by loss of a proton and its replacement on other face of the enamine.

So here is the enamine that we are considering.

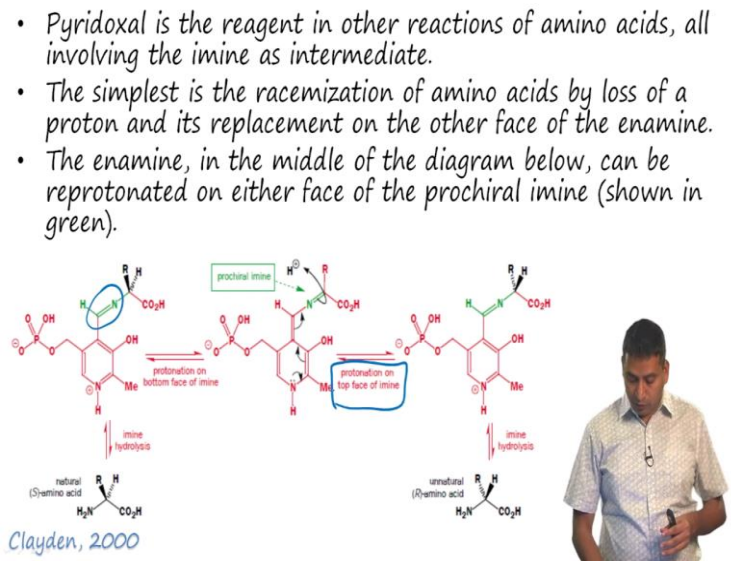
(Refer Slide Time: 08:27)



And as you can think about this is actually giving to be a prochiral imine. And once the imine is going to access or the proton can react with this from the top face or the bottom face you can, you can consider two different isomers of the, enantiomers of the, of the amino acid being formed, right.

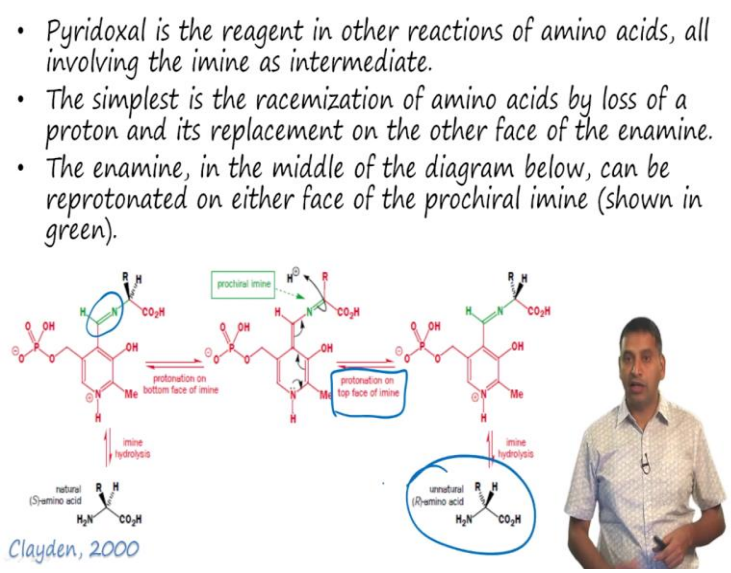
So if the top face undergoes

(Refer Slide Time: 08:49)



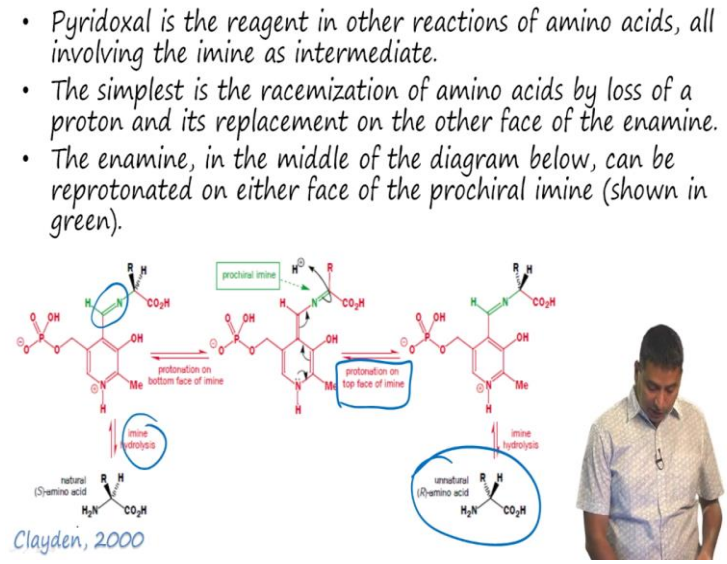
protonation you end up with this

(Refer Slide Time: 08:51)



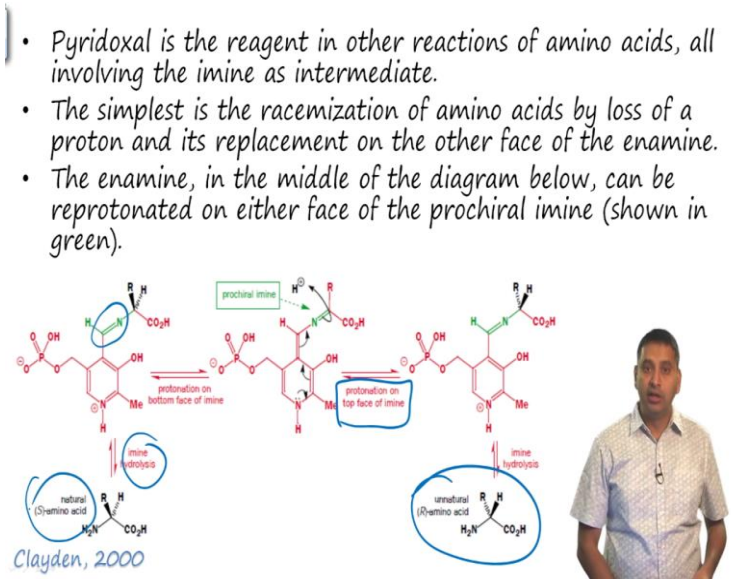
unnatural amino acid as shown here and if the bottom phase undergoes protonation followed by imine

(Refer Slide Time: 08:58)



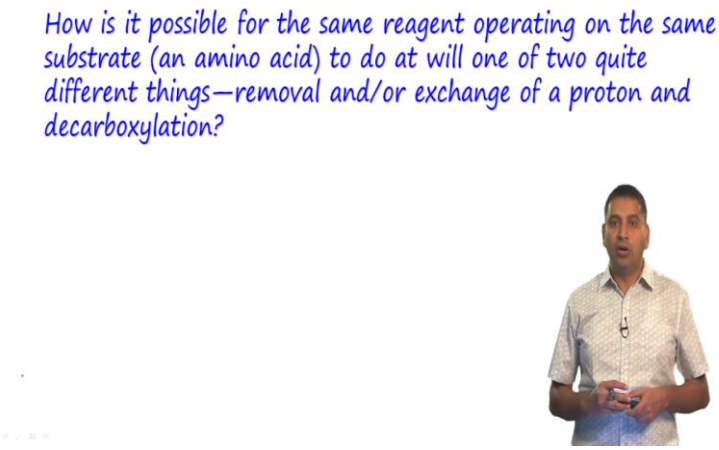
hydrolysis you actually end up with the

(Refer Slide Time: 09:00)



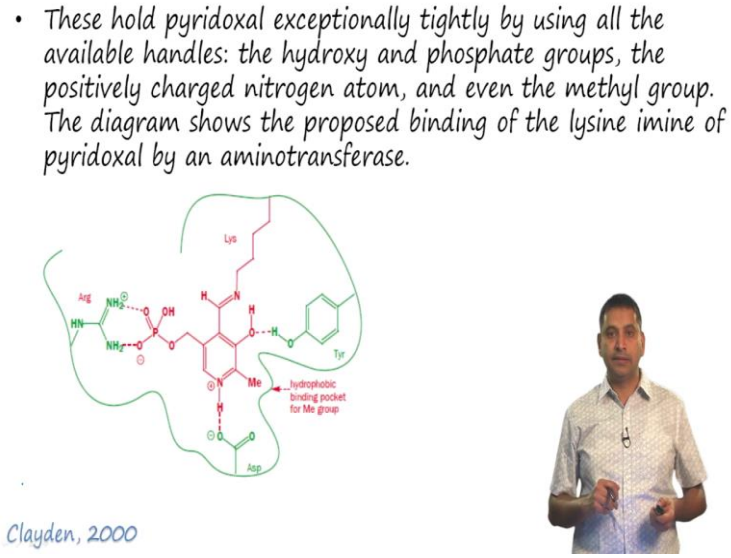
natural amino acid as shown here.

(Refer Slide Time: 09:03)



So how is it possible for the same reagent operating on the same substrate that is an amino acid to do at will, one of the two quite different things, that is one is removal of a proton and another one is decarboxylation

(Refer Slide Time: 09:21)

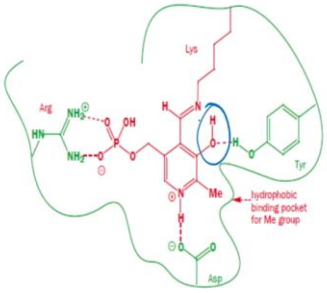


So in order to understand this we shall look at the, how the enzyme is positioned, right? So the enzyme or the protein is exceptionally, is very tightly bound in all handles, right.

So for example this OH here

(Refer Slide Time: 09:39)

- These hold pyridoxal exceptionally tightly by using all the available handles: the hydroxy and phosphate groups, the positively charged nitrogen atom, and even the methyl group. The diagram shows the proposed binding of the lysine imine of pyridoxal by an aminotransferase.

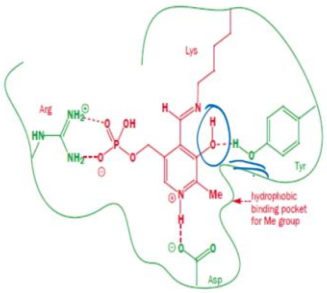


Clayden, 2000

is bound to a tyrosine residue as shown here.

(Refer Slide Time: 09:41)

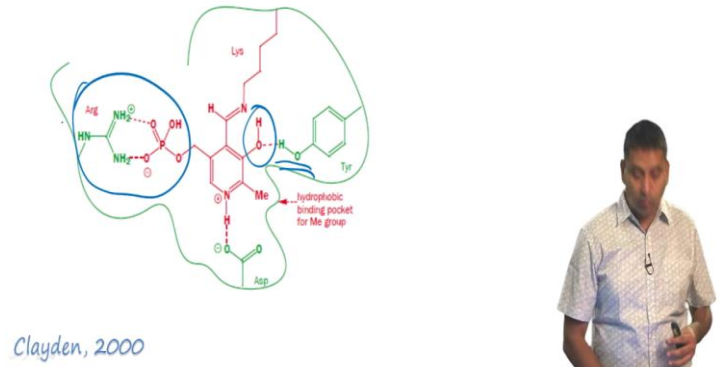
- These hold pyridoxal exceptionally tightly by using all the available handles: the hydroxy and phosphate groups, the positively charged nitrogen atom, and even the methyl group. The diagram shows the proposed binding of the lysine imine of pyridoxal by an aminotransferase.



Clayden, 2000

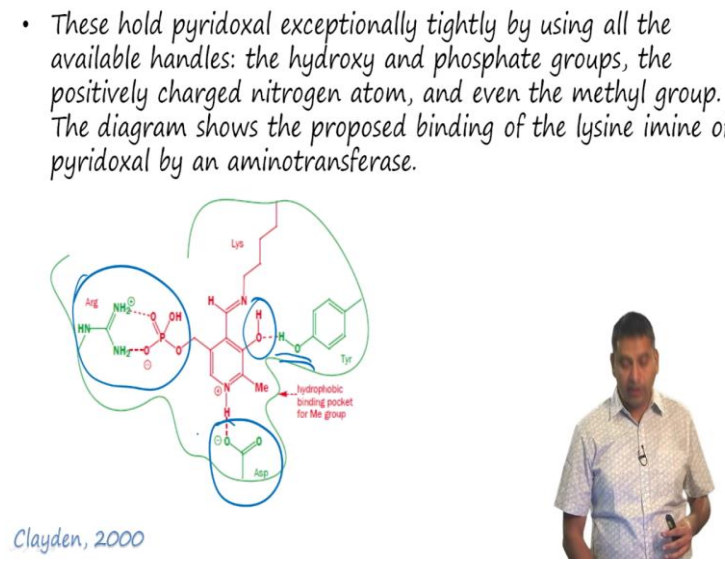
The phosphate is bound

(Refer Slide Time: 09:44)



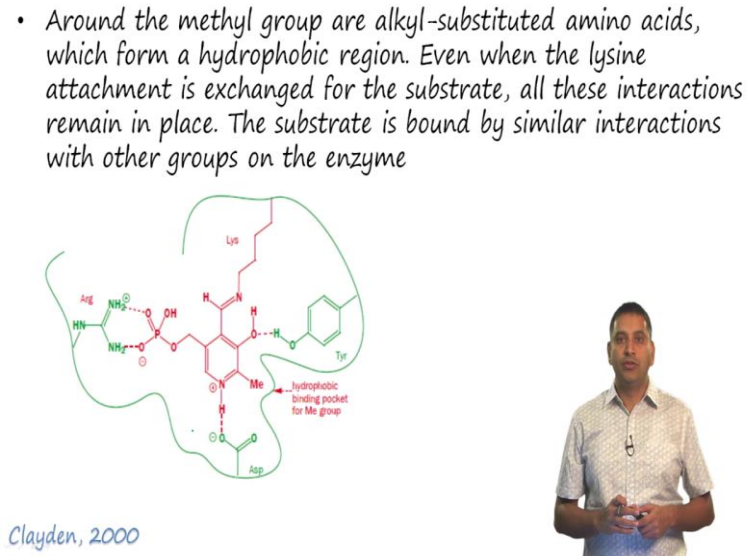
to arginine, Ok and you have an aspartic or

(Refer Slide Time: 09:48)



aspartate reacting with the, or interacting with the positively charged pyridinium, right. So this holds the substrate in a very tight manner.

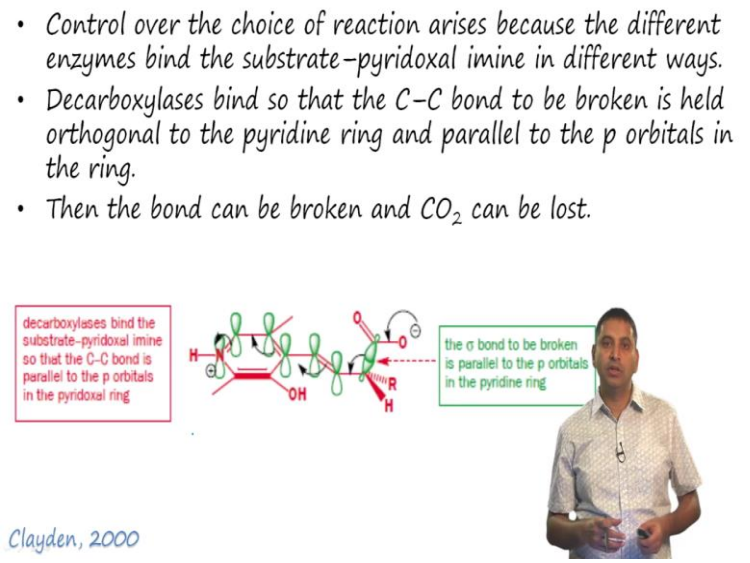
(Refer Slide Time: 09:58)



Ok.

So then around the methyl group there are alkyl substituted amino acids which form a hydrophobic region. And even when the lysine attachment is exchanged for the substrate, all these interactions remain in place. So therefore the substrate is bound by similar interactions with other groups on the enzyme.

(Refer Slide Time: 10:17)

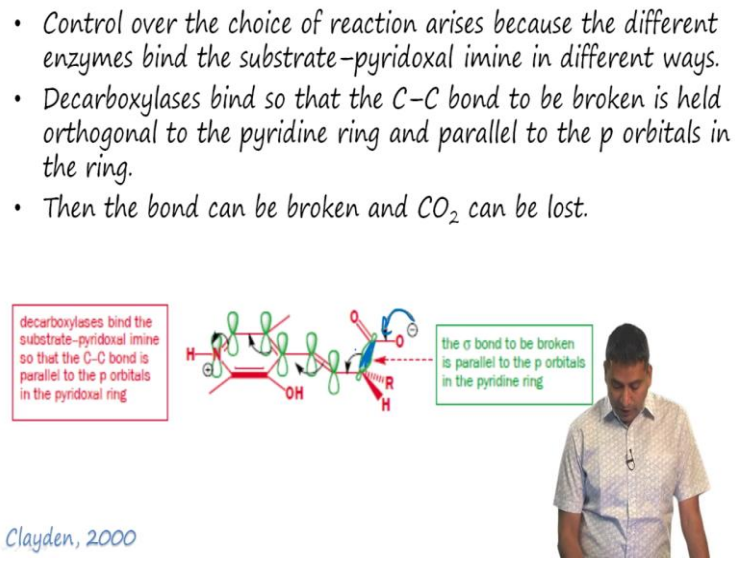


Now control over the choice of reaction arises because the different enzymes bind the substrate pyridoxal imine in different ways. So decarboxylases are enzymes which bind such that the carbon carbon bond that is to be broken is held orthogonal to the pyridine ring and parallel to the p orbitals in the ring.

So if decarboxylation were to occur so this is the key bond that has to break. And this is held perpendicular or orthogonal to the olefin, right.

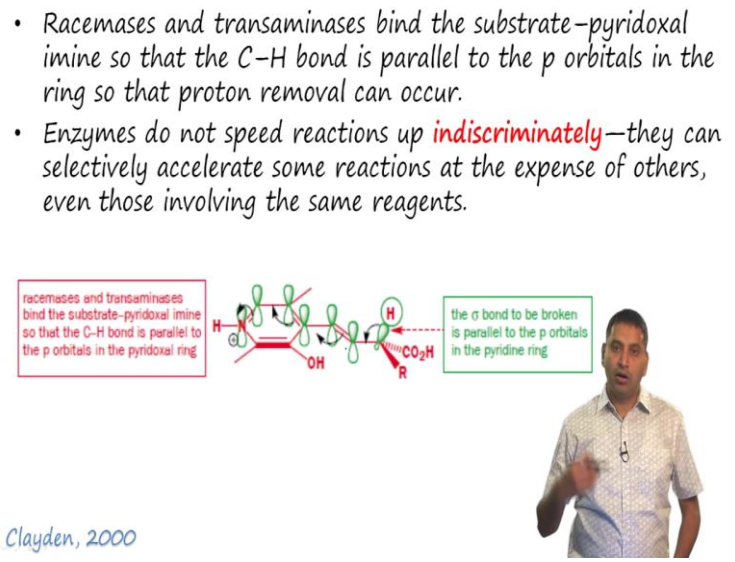
Now you can push arrows and

(Refer Slide Time: 10:52)



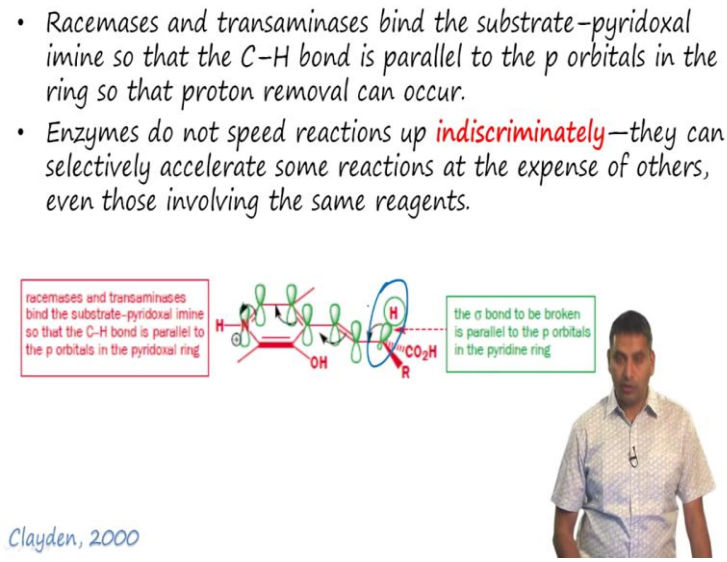
it is going to result in, in the decarboxylation reaction and carbon dioxide can be lost.

(Refer Slide Time: 10:59)



Alternatively if you want deprotonation to occur, then the CH is actually held orthogonal

(Refer Slide Time: 11:07)



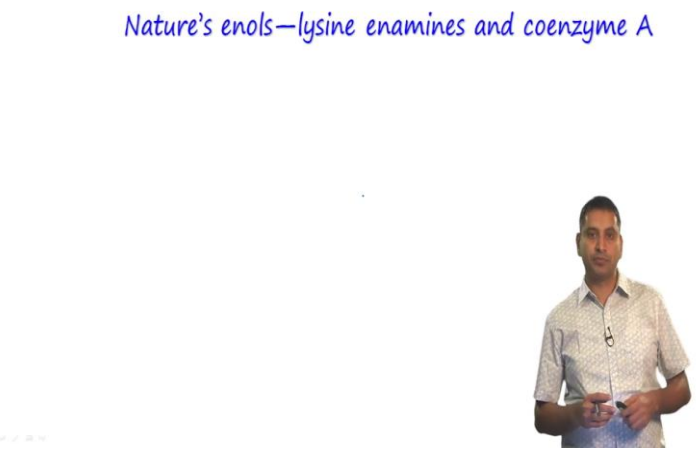
to this. So enzymes do not speed up reactions indiscriminately.

We have already looked that in detail how enzymes are able to accelerate reactions and they, what they do is that they selectively accelerate some reactions at the expense of others, Ok.

So therefore in this very simple example, what we have looked at is the positioning of the bond that needs to be broken is very important in determining the outcome of the reaction. And certain enzymes can stabilize this carbon hydrogen bond breaking or the transition state leading to that, whereas there are other enzymes which can actually catalyze the decarboxylation.

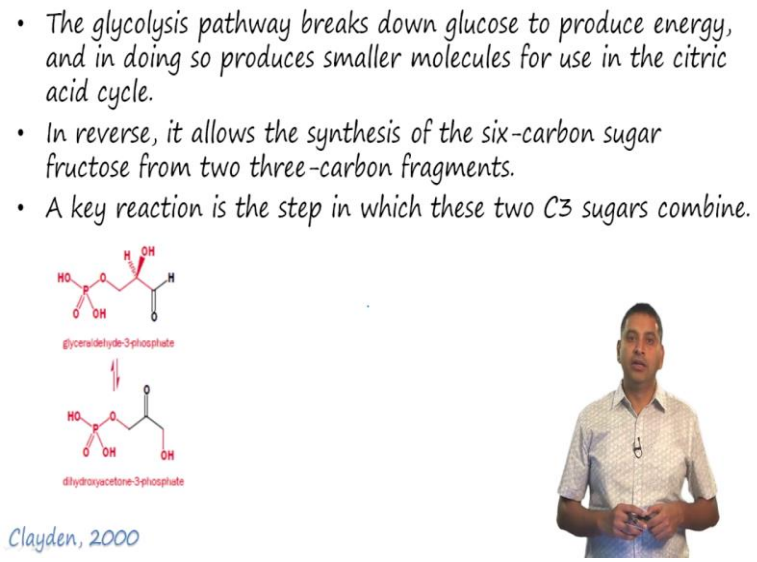
So this is a beautiful example about how, by just doing a simple carbon carbon bond rotation we are able to access two different products.

(Refer Slide Time: 11:59)



The next topic we are going to look at is nature's enols, which is lysine enamines and coenzyme A, Ok.

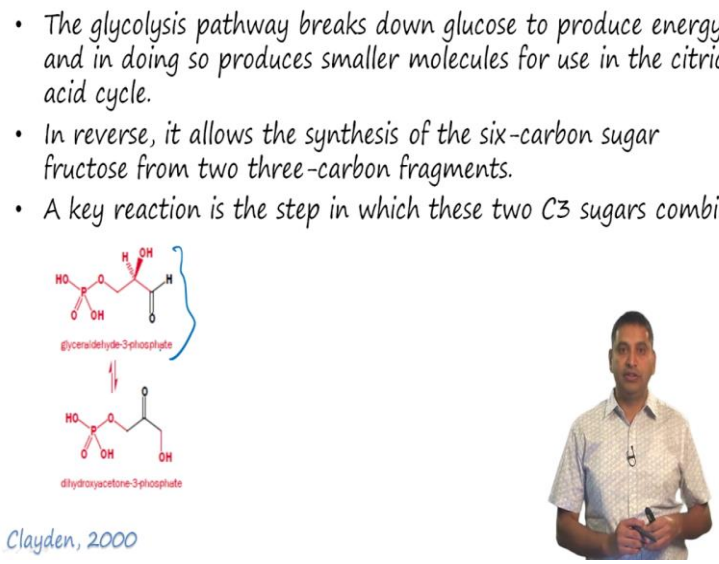
(Refer Slide Time: 12:09)



So the glycolysis pathway breaks down glucose to produce energy. We have already looked at this previously. And in doing so it produces smaller molecules for use in the citric acid cycle. In the reverse process, it allows the synthesis of six-carbon sugars such as fructose from three-carbon fragments.

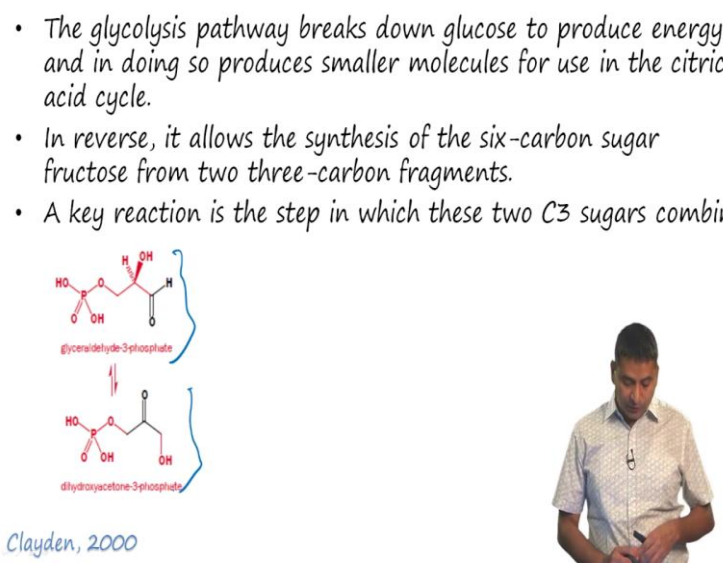
A key reaction in this step is to, is with two C3 carbon sugars to combine. So what the process that we are looking at

(Refer Slide Time: 12:37)



is basically glyceraldehydes-3-phosphate is going to produce

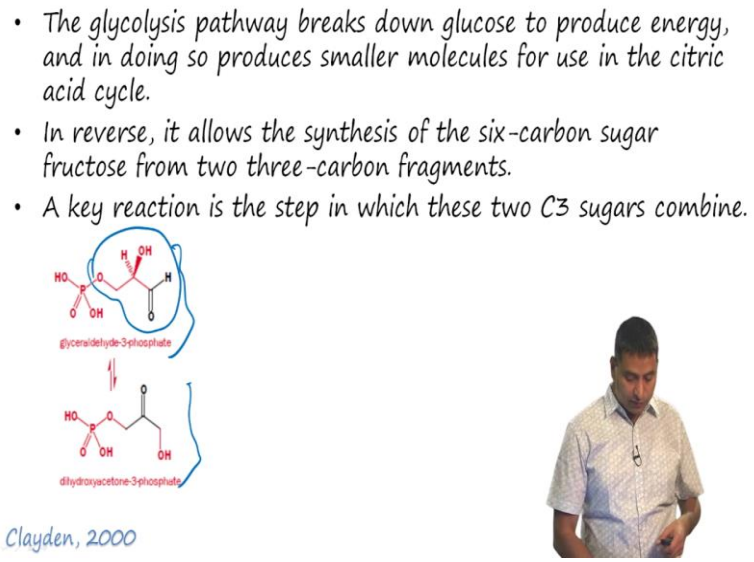
(Refer Slide Time: 12:41)



dihydroxy-acetone-3-phosphate, Ok.

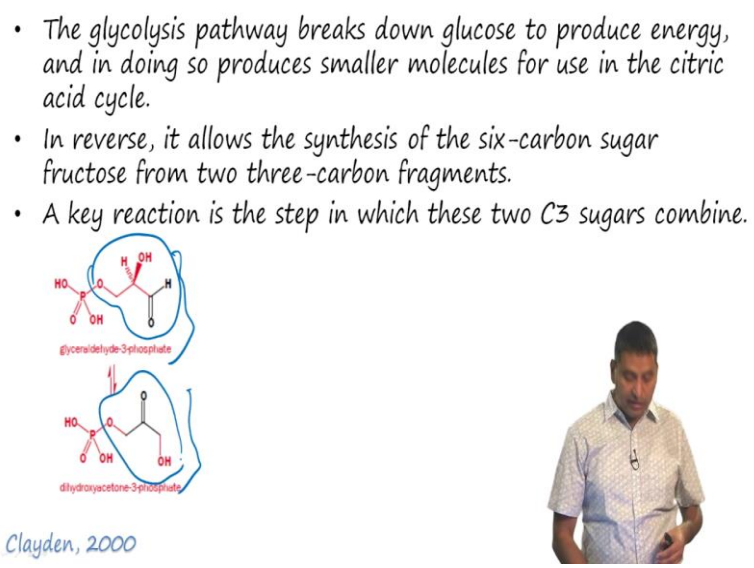
So glyceraldehyde is shown here

(Refer Slide Time: 12:49)



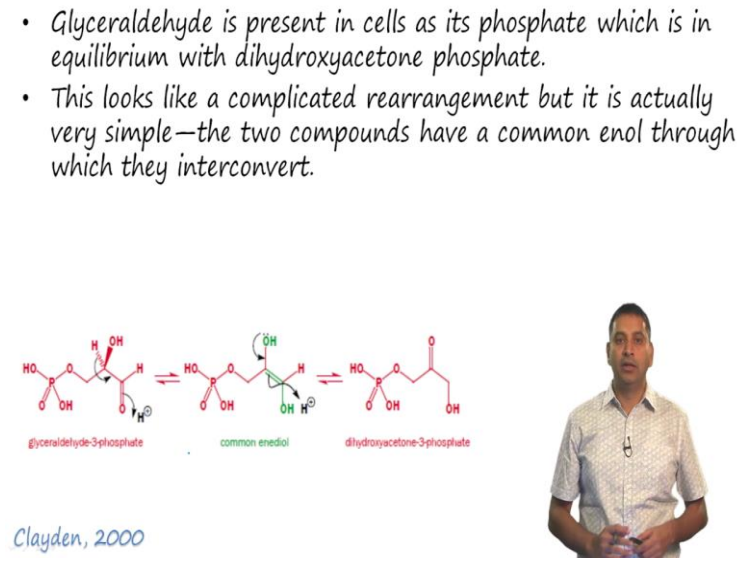
which is basically the aldehyde or the oxidized form of glycerol. And dihydroxy-acetone-phosphate is shown here which is basically

(Refer Slide Time: 12:57)



the oxidation, the carbonyl is shifted to the internal carbon.

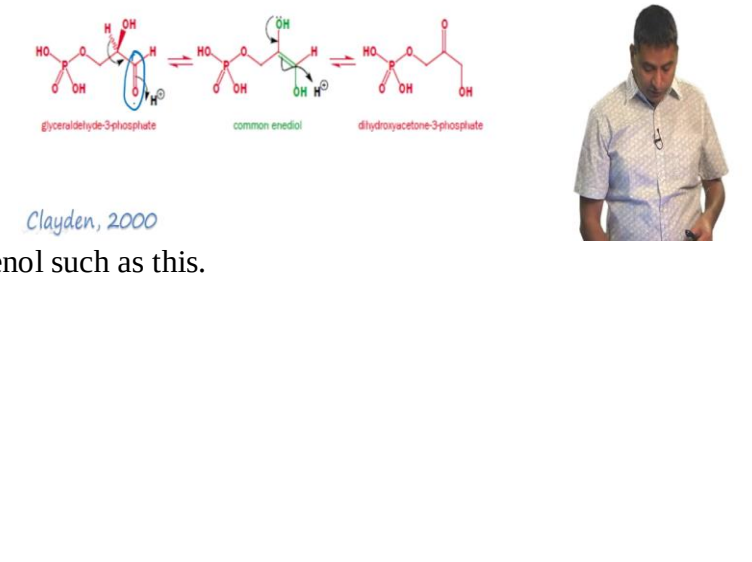
(Refer Slide Time: 13:03)



Now glyceraldehyde is present in cells as its phosphate which is in equilibrium with dihydroxyacetone phosphate. This looks like a very complicated rearrangement but actually it is a very simple one where the two compounds have a common enol which then interconverts.

So if this aldehyde enolizes

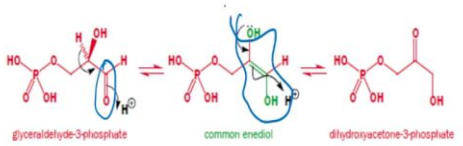
(Refer Slide Time: 13:24)



it produces an enol such as this.

(Refer Slide Time: 13:27)

- Glyceraldehyde is present in cells as its phosphate which is in equilibrium with dihydroxyacetone phosphate.
- This looks like a complicated rearrangement but it is actually very simple—the two compounds have a common enol through which they interconvert.

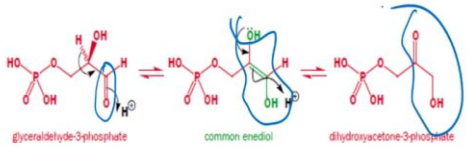


Clayden, 2000

Then you can push arrows to give you the corresponding

(Refer Slide Time: 13:31)

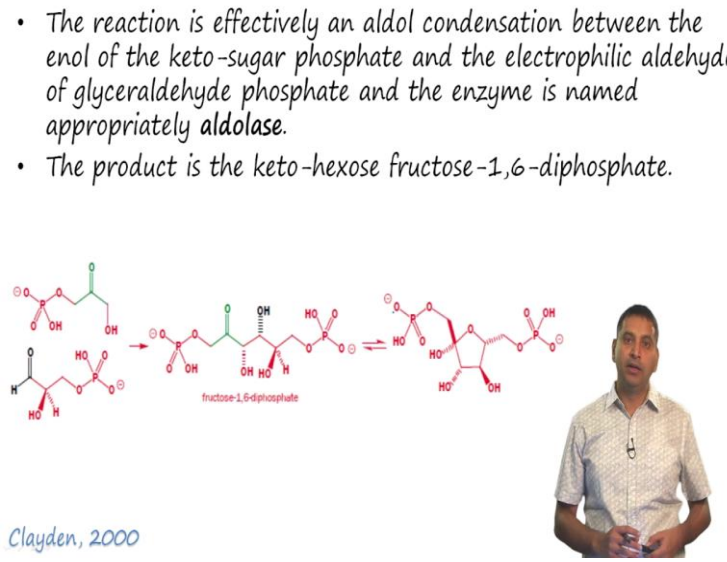
- Glyceraldehyde is present in cells as its phosphate which is in equilibrium with dihydroxyacetone phosphate.
- This looks like a complicated rearrangement but it is actually very simple—the two compounds have a common enol through which they interconvert.



Clayden, 2000

dihydroxyacetone phosphate.

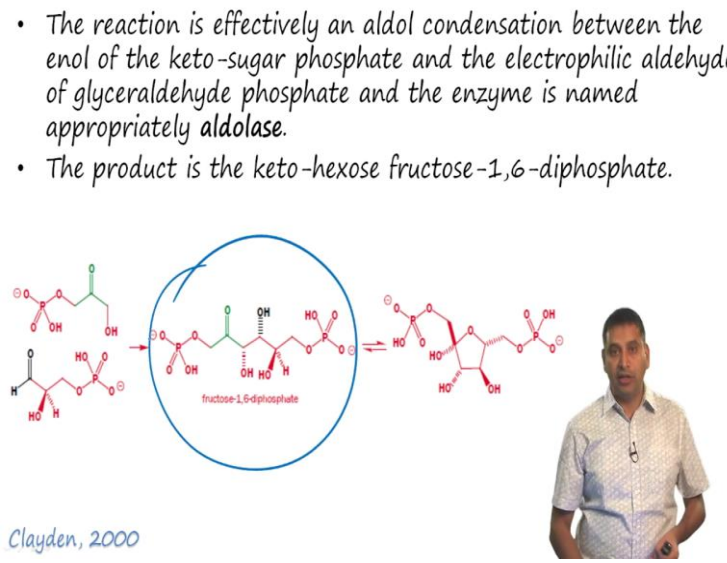
(Refer Slide Time: 13:34)



The reaction is effectively an aldol condensation between the enol of the keto sugar phosphate and the electrophilic aldehyde of, this is an glyceraldehyde phosphate and the enzyme is known as aldolase, Ok.

So these two units are going to combine to give you a 6 carbon unit, right. And this product is the keto hexose fructose-1, 6-diphosphate which is shown here which then

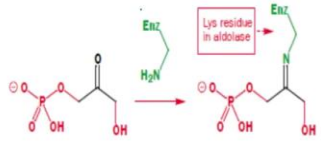
(Refer Slide Time: 14:03)



equilibrates to give you the close ribose form.

(Refer Slide Time: 14:07)

- No enolate ion is formed in this aldol. Instead a lysine residue in the enzyme forms an imine with the keto-triose.
- Proton transfers allow this imine to be converted into an enamine, which acts as the nucleophile in the aldol reaction.



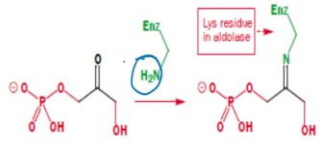
Clayden, 2000

So no enolate is formed in this aldol but instead a lysine residue in the enzyme helps in formation of an imine with the keto triose.

Let us look at this reaction now. So once lysine is going to interact

(Refer Slide Time: 14:24)

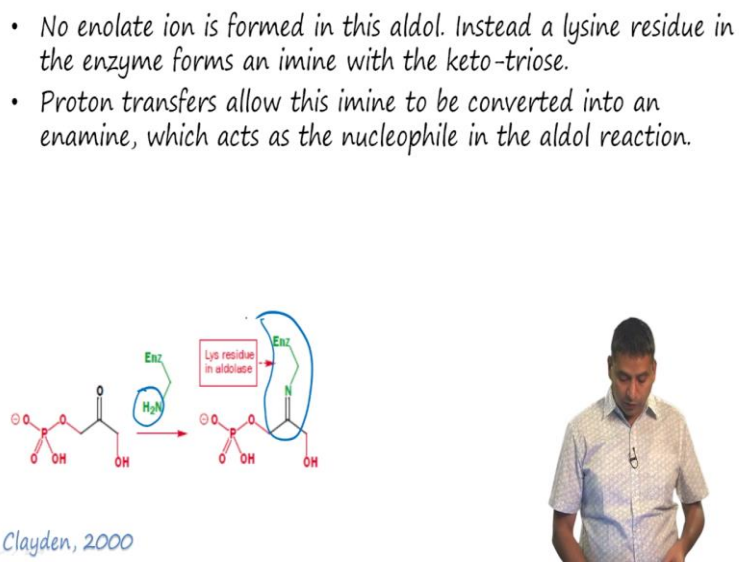
- No enolate ion is formed in this aldol. Instead a lysine residue in the enzyme forms an imine with the keto-triose.
- Proton transfers allow this imine to be converted into an enamine, which acts as the nucleophile in the aldol reaction.



Clayden, 2000

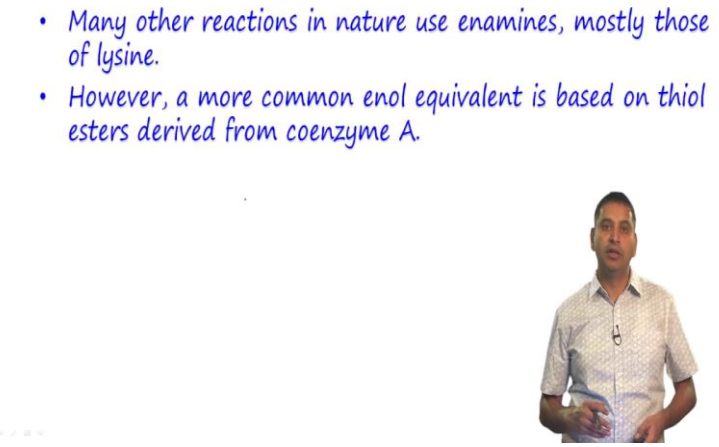
with this carbonyl this is going to form this kind of

(Refer Slide Time: 14:27)



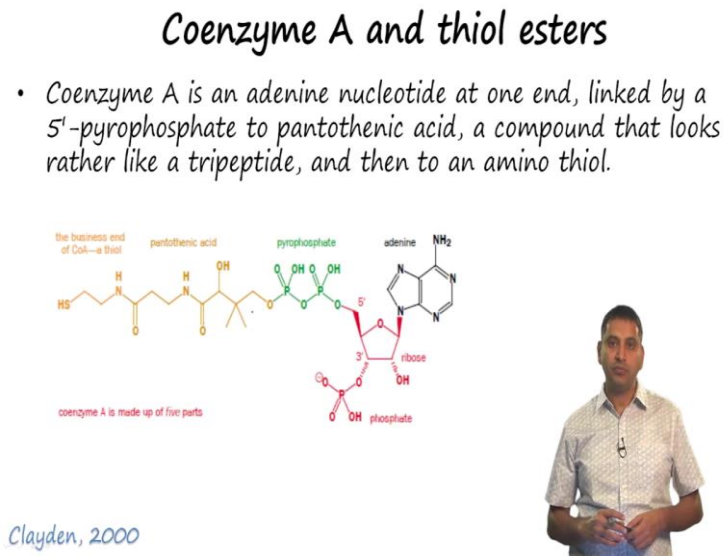
an imine, right. And then this can happen through proton transfer and they allow this imine to be converted to an enamine which then acts as the nucleophile in the aldol reaction.

(Refer Slide Time: 14:38)



So many of the reactions in nature use enamines mostly those of lysine. However a more common enol equivalent is based on thiol esters derived from coenzyme A.

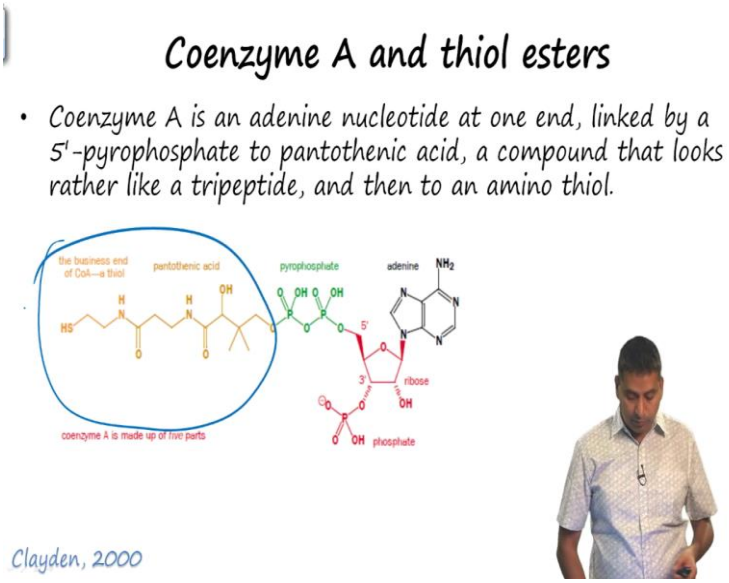
(Refer Slide Time: 14:52)



So coenzyme A is basically an adenine nucleotide on one end and it is linked by a 5 prime pyrophosphate to pantothenic acid, Ok. So pantothenic acid looks a lot, is basically like a, is a tripeptide and that is attached to an amino thiol.

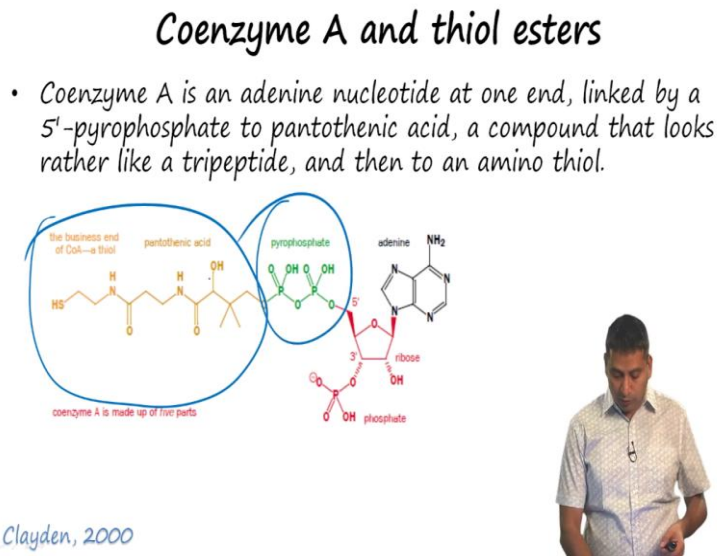
So let us look at this, this molecule in detail. So here is the tripeptide

(Refer Slide Time: 15:17)



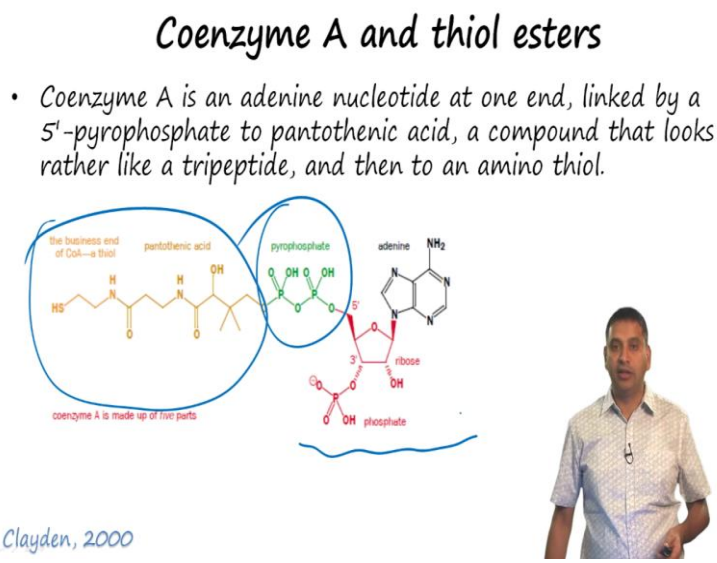
which is pantothenic acid. Here is the

(Refer Slide Time: 15:19)



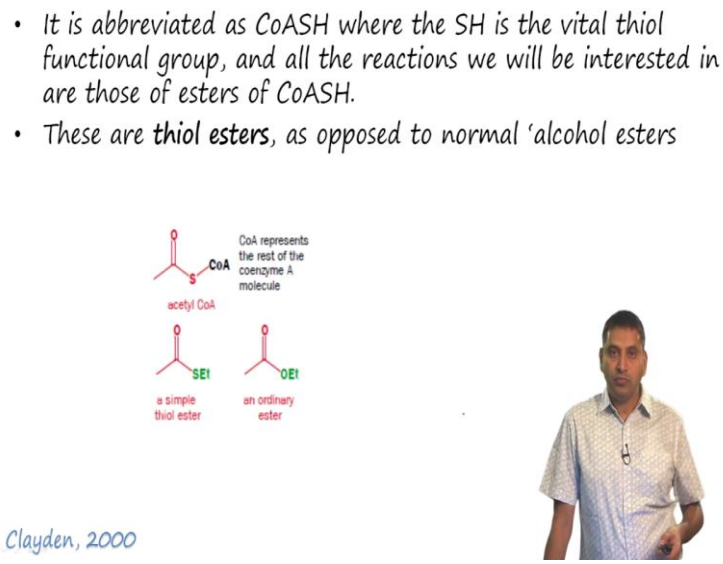
pyrophosphate group and here is

(Refer Slide Time: 15:21)



the nucleo base and the ribose,

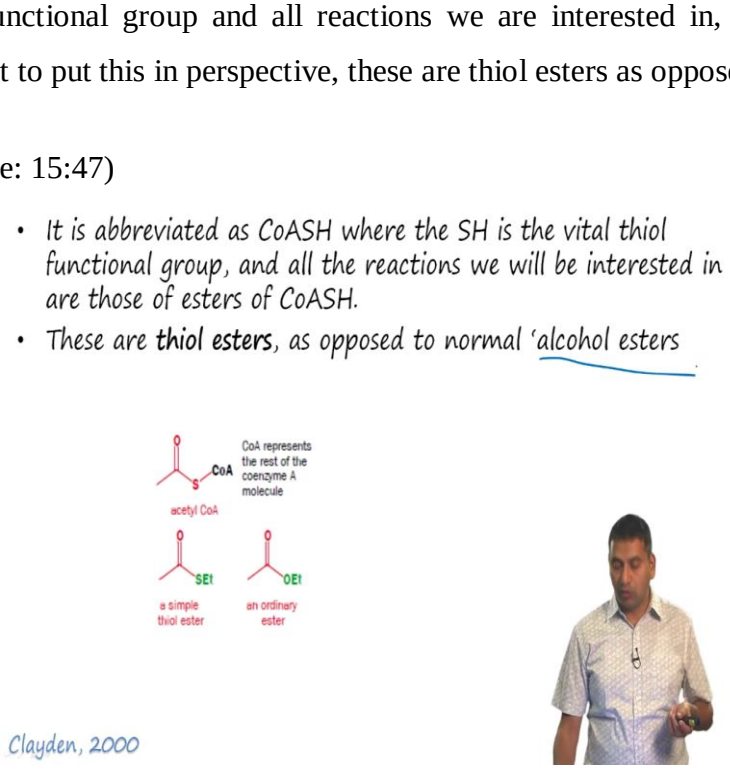
(Refer Slide Time: 15:26)



Ok.

So this molecule is actually abbreviated as CoASH as Co A thiol. And when SH, this SH is the vital thiol functional group and all reactions we are interested in, will be that of the CoASH. And just to put this in perspective, these are thiol esters as opposed to the normal

(Refer Slide Time: 15:47)

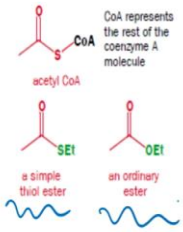


alcohol esters that we are used to.

So this is the thiol ester

(Refer Slide Time: 15:52)

- It is abbreviated as CoASH where the SH is the vital thiol functional group, and all the reactions we will be interested in are those of esters of CoASH.
- These are **thiol esters**, as opposed to normal 'alcohol esters



Clayden, 2000

and this is the ordinary ester that we are more used to.

(Refer Slide Time: 15:56)

- Thiol esters are less conjugated than ordinary esters, and ester hydrolysis occurs more rapidly with thiol esters than with ordinary esters because in the rate-determining step (nucleophilic attack on the carbonyl group) there is less conjugation to destroy.
- The thiolate is also a better leaving group.

Clayden, 2000

Thiol esters interestingly are less conjugated than ordinary esters, Ok. So this is because the sulphur, the overlap between the sulphur and carbonyl or the interaction between the sulphur and carbonyl is not as strong as between oxygen and the carbonyl.

And so the ester hydrolysis occurs more rapidly with thiol esters than with ordinary esters. Because in the rate determining step there is less conjugation to destroy. Also the thiolate is a much better leaving group.

(Refer Slide Time: 16:29)

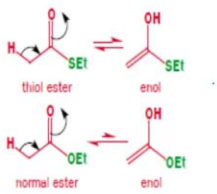
- Thiol esters are less conjugated than ordinary esters, and ester hydrolysis occurs more rapidly with thiol esters than with ordinary esters because in the rate-determining step (nucleophilic attack on the carbonyl group) there is less conjugation to destroy.
- The thiolate is also a better leaving group.

Clayden, 2000

We already looked at pKa's of various thiols and thiols are much better acids compared to alcohols.

(Refer Slide Time: 16:37)

- Another reaction that goes better with thiol esters than with ordinary esters is enolization.
- This is an equilibrium reaction and the enol has lost the conjugation present in the ester.
- The thiol ester has less to lose so is more enolized.

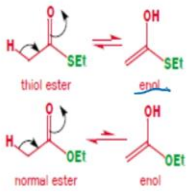


Clayden, 2000

So another reaction that goes better with thiol esters than with ordinary esters is enolization. So this is an equilibrium which we have looked at previously where the keto exists as an equilibrium with the enol, right. So the thioester similarly exists in its enol form.

(Refer Slide Time: 16:56)

- Another reaction that goes better with thiol esters than with ordinary esters is enolization.
- This is an equilibrium reaction and the enol has lost the conjugation present in the ester.
- The thiol ester has less to lose so is more enolized.

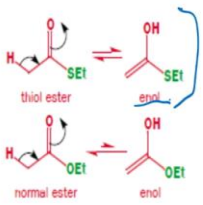


Clayden, 2000

And if we were to draw the enol form of the thiol, it should look like this.

(Refer Slide Time: 17:00)

- Another reaction that goes better with thiol esters than with ordinary esters is enolization.
- This is an equilibrium reaction and the enol has lost the conjugation present in the ester.
- The thiol ester has less to lose so is more enolized.

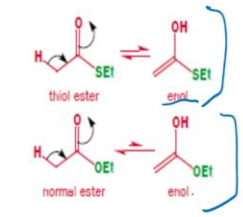


Clayden, 2000

Whereas with a normal ester, the enol is going to look

(Refer Slide Time: 17:04)

- Another reaction that goes better with thiol esters than with ordinary esters is enolization.
- This is an equilibrium reaction and the enol has lost the conjugation present in the ester.
- The thiol ester has less to lose so is more enolized.



Clayden, 2000

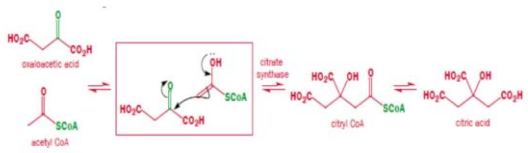
like this. So a thiol ester has less to lose and so it is generally more enolized, Ok.

So again the equilibrium between the normal ester and thiol ester are going to be different and the normal ester is far less enolized compared to a thioester. So together what we can understand is that a thioester can be hydrolized more rapidly and this is attributed to how the carbonyl is more reactive and also the thiolate being a better leaving group.

Also the thioester is more in the enol form compared to the normal ester. So therefore this becomes an ideal vehicle for an enolate transfer.

(Refer Slide Time: 17:50)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.

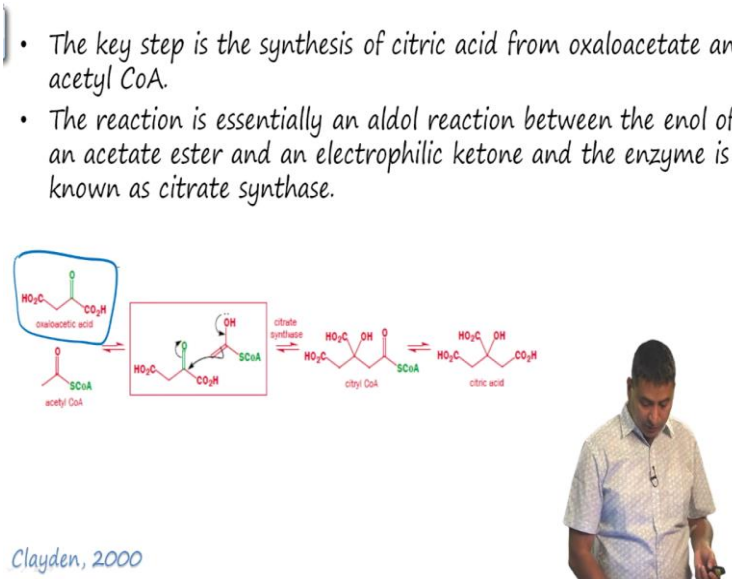


Clayden, 2000

So the key step in the synthesis of citric acid from oxaloacetate as we have looked at previously is from oxaloacetate and acetyl CoA.

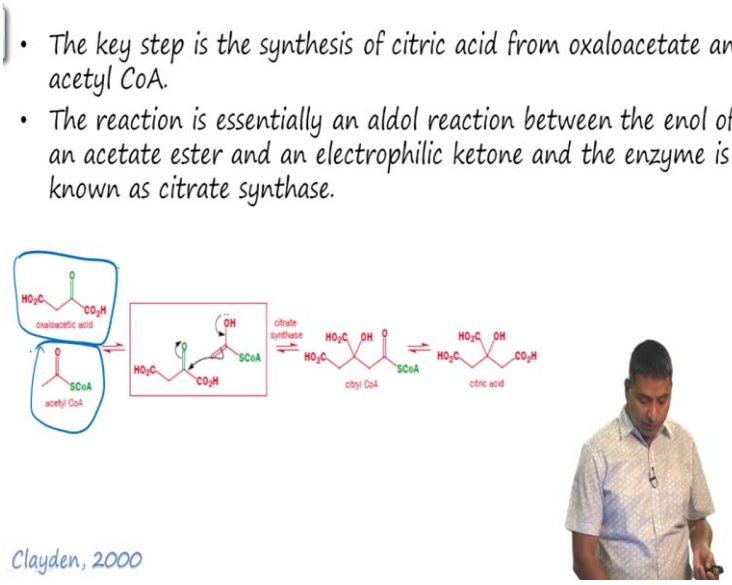
So the reaction is essentially an aldol reaction between the enol of an acetate ester which is shown here,

(Refer Slide Time: 18:10)



Ok and the reaction between these two,

(Refer Slide Time: 18:13)

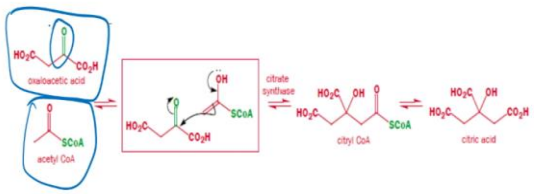


which is oxaloacetic acid and acetyl CoA.

Now the oxaloacetic acid has an electrophilic ketone which is shown here,

(Refer Slide Time: 18:23)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.



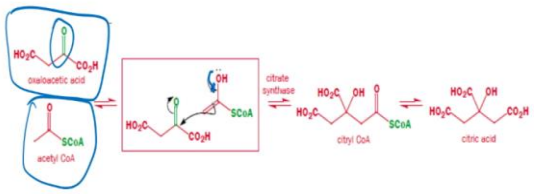
Clayden, 2000

right and this is the ketone that is going to react. So acetyl CoA can exist in its enol form and we have already looked at the equilibrium is going to be favoring the enol.

So if you push arrows from here, you end up

(Refer Slide Time: 18:39)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.

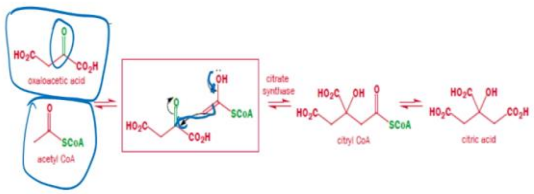


Clayden, 2000

with this reaction where

(Refer Slide Time: 18:41)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.

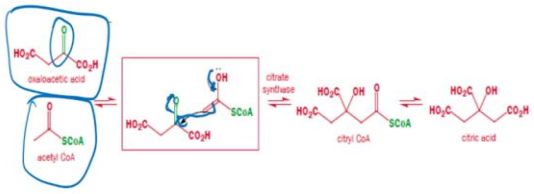


Clayden, 2000

the enolate

(Refer Slide Time: 18:42)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.

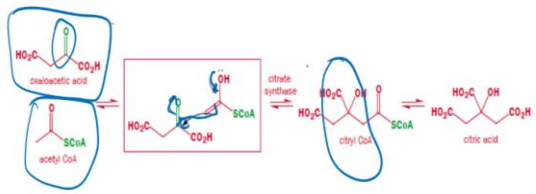


Clayden, 2000

is going to react and give you this citryl CoA,

(Refer Slide Time: 18:45)

- The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA.
- The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as citrate synthase.

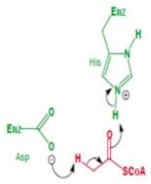


Clayden, 2000

right. And subsequently loss of the Co A can give you the corresponding citric acid.

(Refer Slide Time: 18:52)

- In nature the **enolization** is catalysed by a basic carboxylate group (Asp) and an acidic histidine, both part of the enzyme, so that even this easy reaction goes faster.

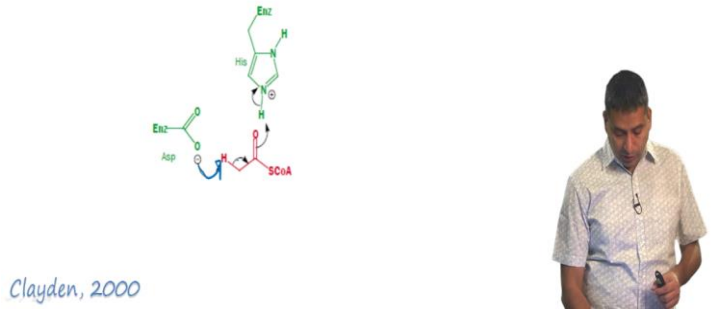


Clayden, 2000

So in nature, enolization is catalyzed by a basic carboxylate group such as aspartate and an acidic histidine, we have already looked that in detail about histidine, how histidine can act as a proton donor as well as a proton acceptor. And both are part of the enzyme.

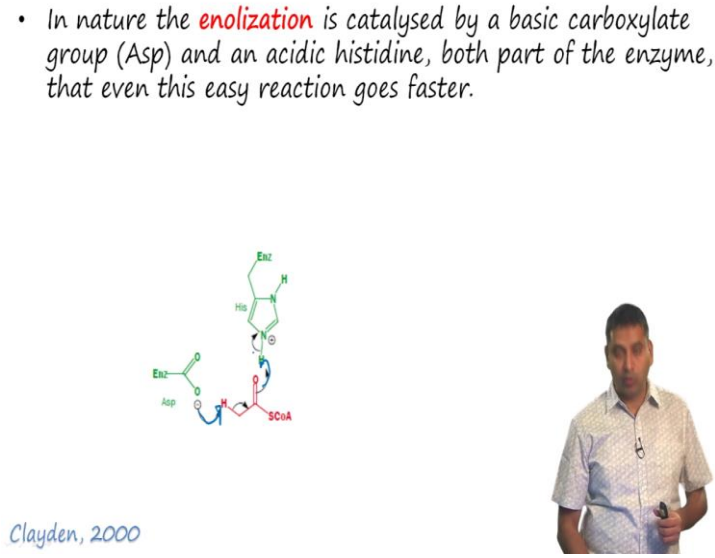
And so this makes the reaction go even faster. So aspartate can pick up this proton

(Refer Slide Time: 19:16)



here and push the equilibrium more towards the enolate and this carbonyl can

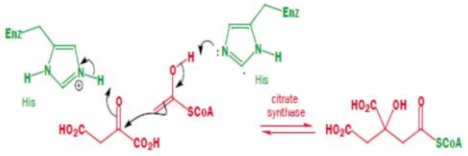
(Refer Slide Time: 19:22)



interact with this proton from histidine and it is going to form the enol.

(Refer Slide Time: 19:27)

- In the C–C bond-forming step, the same histidine is still there to remove the enol proton again and another histidine, in its protonated form, is placed to donate a proton to the oxygen atom of the ketone.
- You should see now why histidine, with a pK_{aH} of about 7, is so useful to enzymes: it can act either as an acid or as a base.



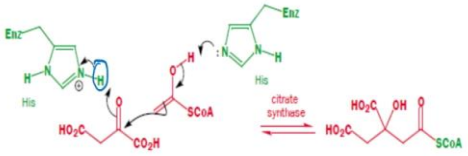
Clayden, 2000

Now this, in the carbon carbon bond forming step, the same histidine is still there to remove the enol proton again and another histidine in its protonated form is placed to donate a proton to the oxygen atom of the ketone.

So here is the histidine

(Refer Slide Time: 19:47)

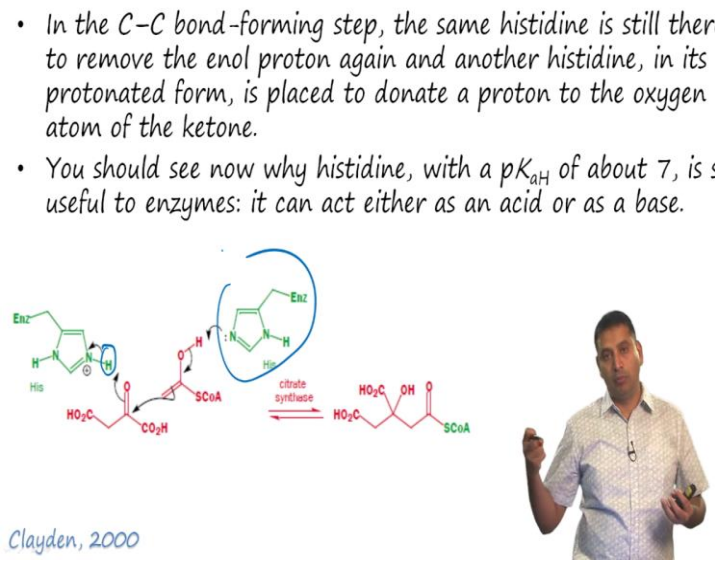
- In the C–C bond-forming step, the same histidine is still there to remove the enol proton again and another histidine, in its protonated form, is placed to donate a proton to the oxygen atom of the ketone.
- You should see now why histidine, with a pK_{aH} of about 7, is so useful to enzymes: it can act either as an acid or as a base.



Clayden, 2000

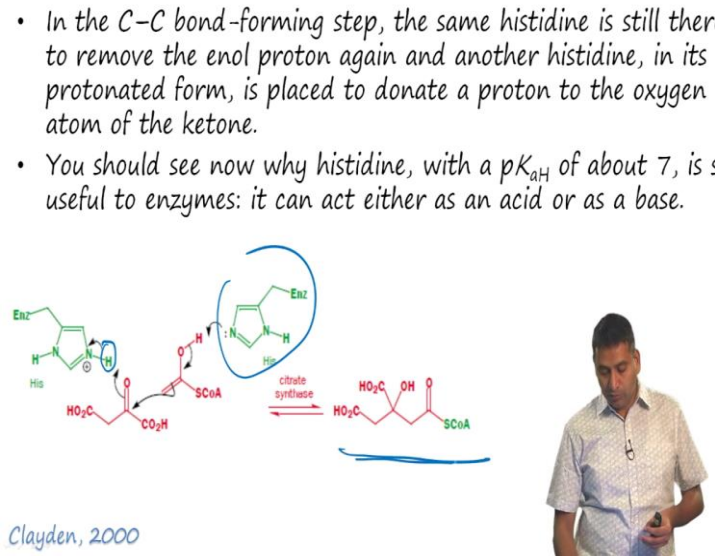
which is going to donate the proton to the ketone and here is the histidine which is going to

(Refer Slide Time: 19:52)



pull put out the proton from the enol. And it is going to give you this

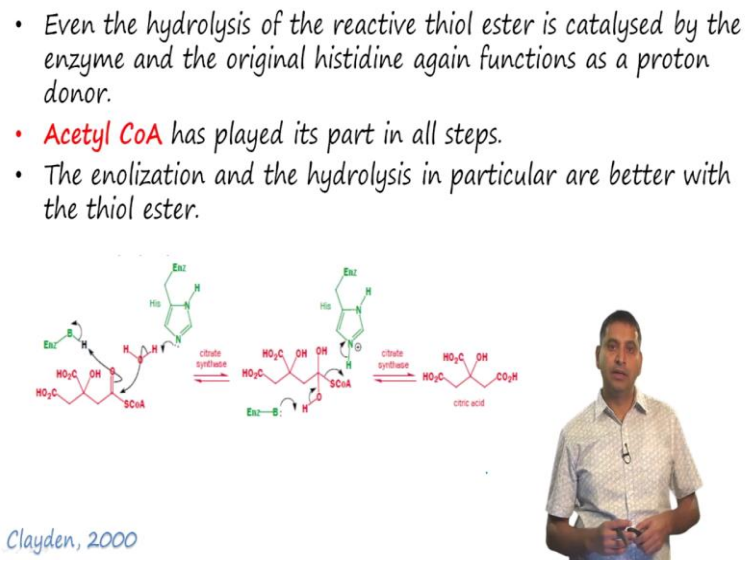
(Refer Slide Time: 19:56)



product here.

Now histidine which has a pKaH of about 7 is so useful in enzymes and it can, because it can act either as a acid or a base,

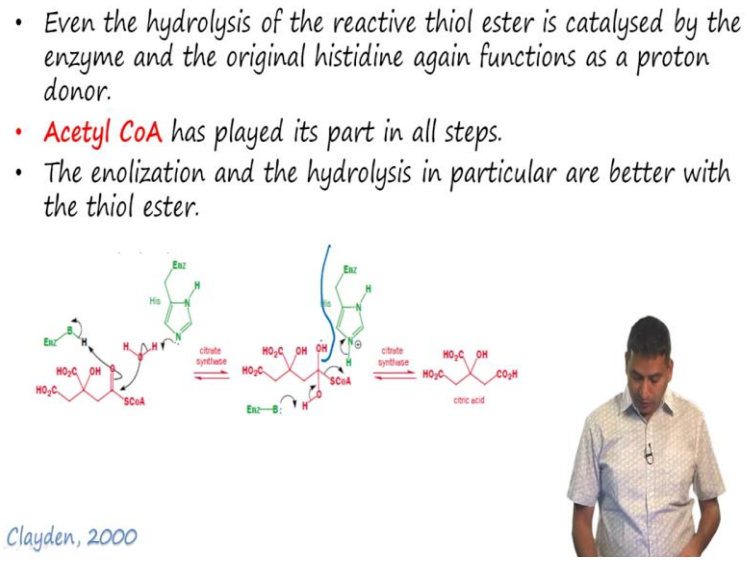
(Refer Slide Time: 20:08)



right. So even the hydrolysis of the reactive thiol ester is catalyzed by the enzyme and original histidine again functions as a proton donor.

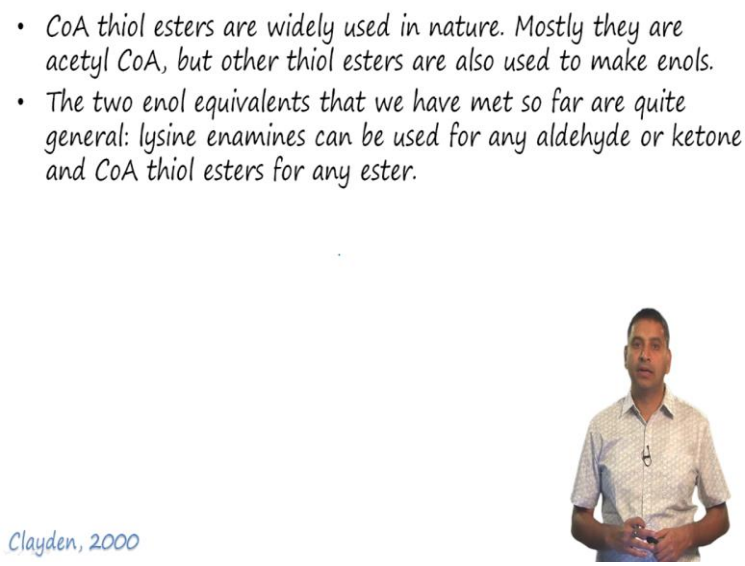
So acetyl CoA has played its parts in all the steps. So this hydrolysis as we have looked at

(Refer Slide Time: 20:25)



right now is also catalyzed by histidine. The enolization and the hydrolysis in particular are better with the thiol ester. So therefore acetyl CoA forms a key, plays a key role in this entire chemistry.

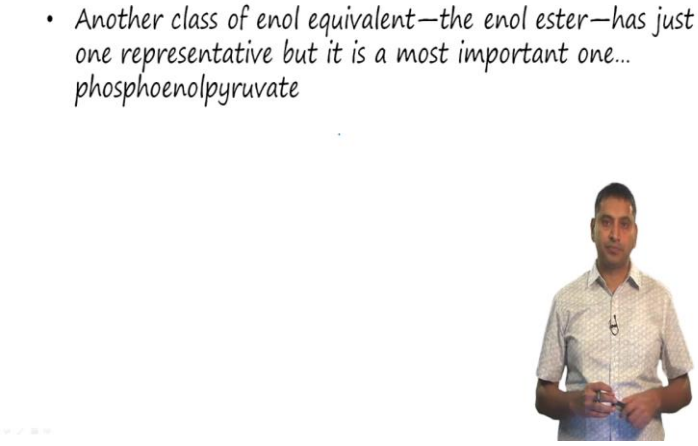
(Refer Slide Time: 20:44)



So CoA thiol esters are widely used in nature. And mostly they are acetyl CoA. But other thiol esters are also used to make enols. The two enol equivalents that we have met so far are quite general, so one is from lysine enamine which can be used for an aldehyde, for any aldehyde or a ketone.

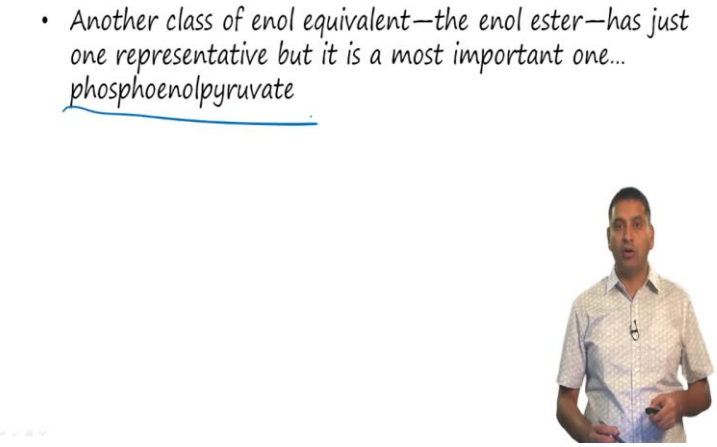
And the other one is a CoA thiol ester which can be used for any ester.

(Refer Slide Time: 21:07)



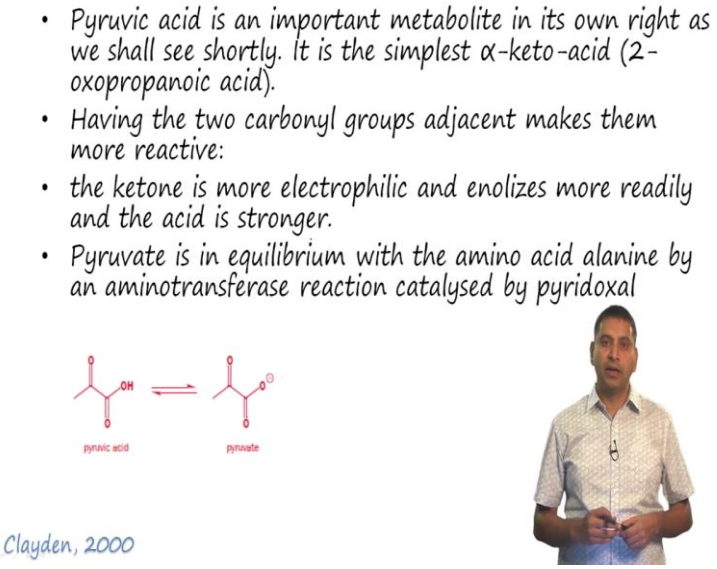
Another class of enol equivalents which is the enol ester has just one representative but it is a very important one and so we are going to look at it. So it is called the

(Refer Slide Time: 21:18)



phosphoenolpyruvate.

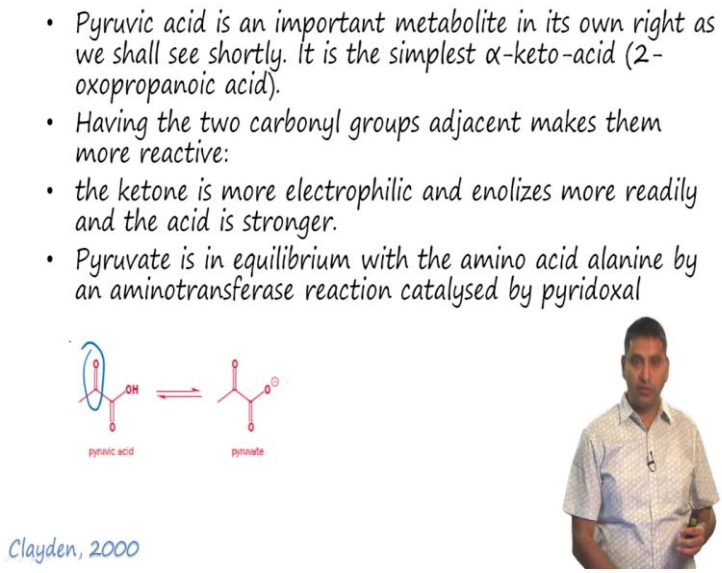
(Refer Slide Time: 21:20)



Pyruvic acid which we have looked at quite a bit of detail in this lecture as well as in the previous lectures is a very important metabolite. Ok and is one of, it is basically the simplest alpha-keto-acid which is basically 2-oxopropanoic acid.

So it has 2 carbonyl groups which are adjacent to one another and makes it quite reactive. So the ketone here is

(Refer Slide Time: 21:44)

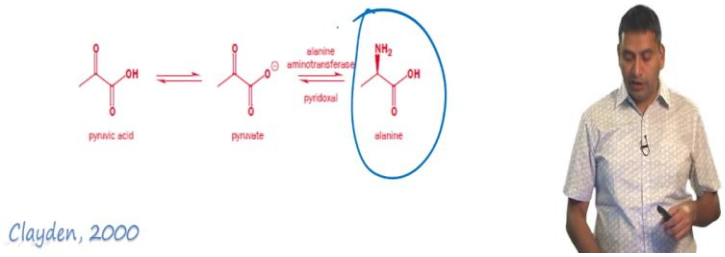


lot more electrophilic compared to a normal ketone and because of this it also enolizes more readily and the acid is much stronger.

So pyruvate is in equilibrium with the amino acid alanine by aminotransferase reaction catalyzed by pyridoxals. We have already looked at how alpha-keto-acids are going to be in the equilibrium with the corresponding amino acids.

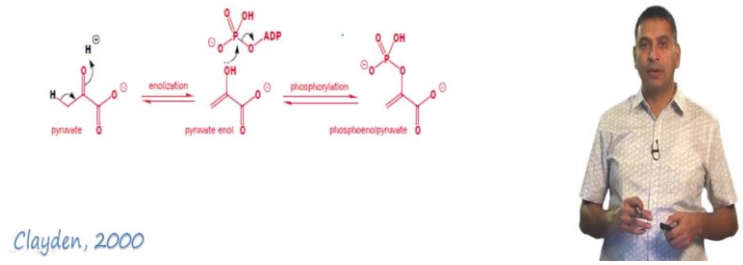
So here is the equilibrium that is shown here.

(Refer Slide Time: 22:11)



So alanine is here and this reaction is catalyzed by pyridoxal.

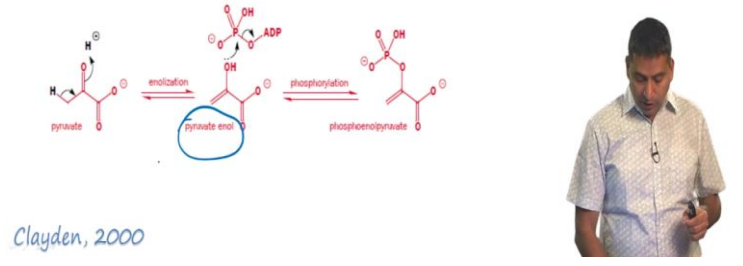
(Refer Slide Time: 22:14)



Nature uses enol phosphate of pyruvic acid which is phosphoenolpyruvate or PEP as an important reagent, Ok. So this can happen by formation of the enol followed by phosphorylation which is mediated by ATP.

So here is the enol of pyruvate, Ok

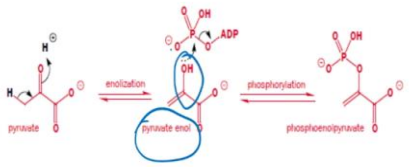
(Refer Slide Time: 22:33)



and since this is an enol

(Refer Slide Time: 22:35)

- Nature uses the enol phosphate of pyruvic acid (**phosphoenolpyruvate** or **PEP**) as an important reagent.
- This can happen by formation of the enol followed by phosphorylation mediated by ATP

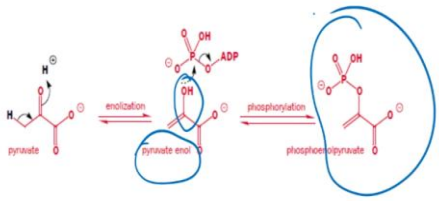


Clayden, 2000

we have already looked at phosphorylation reactions which can occur on OH sites and so this phosphorylation mediated by ATP is going to give me

(Refer Slide Time: 22:43)

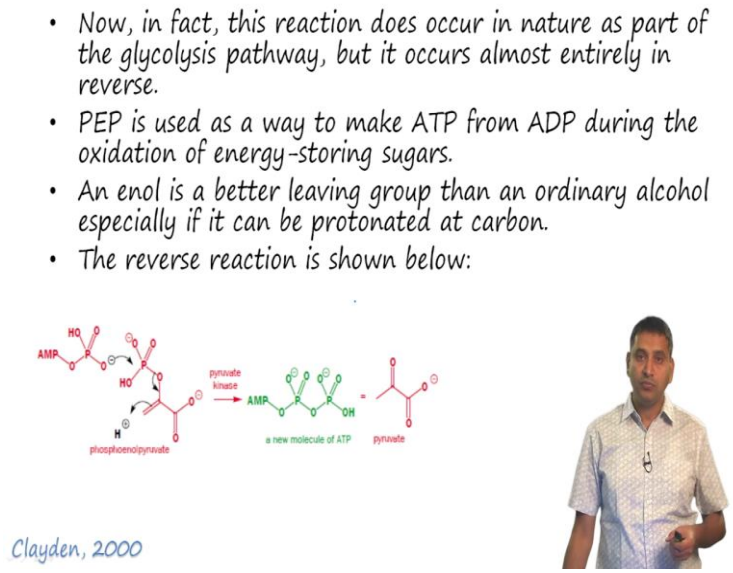
- Nature uses the enol phosphate of pyruvic acid (**phosphoenolpyruvate** or **PEP**) as an important reagent.
- This can happen by formation of the enol followed by phosphorylation mediated by ATP



Clayden, 2000

phosphoenolpyruvate.

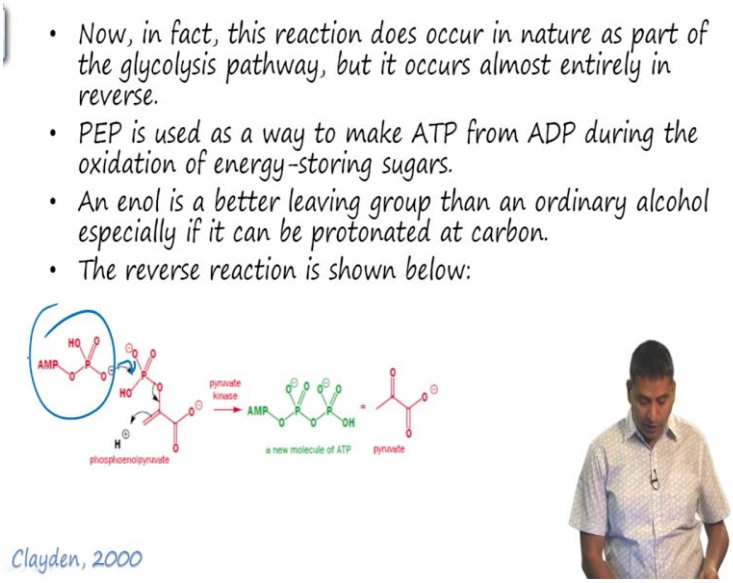
(Refer Slide Time: 22:46)



Now in fact this reaction does not occur in nature as part of the glycolysis pathway but it occurs almost entirely in reverse. This phosphoenolpyruvate is used as a way to make ATP from ADP during the oxidation of energy storing sugars. So an enol is a better leaving group compared to ordinary alcohol especially if it can be protonated with the carbon. So the reverse reaction is shown below here.

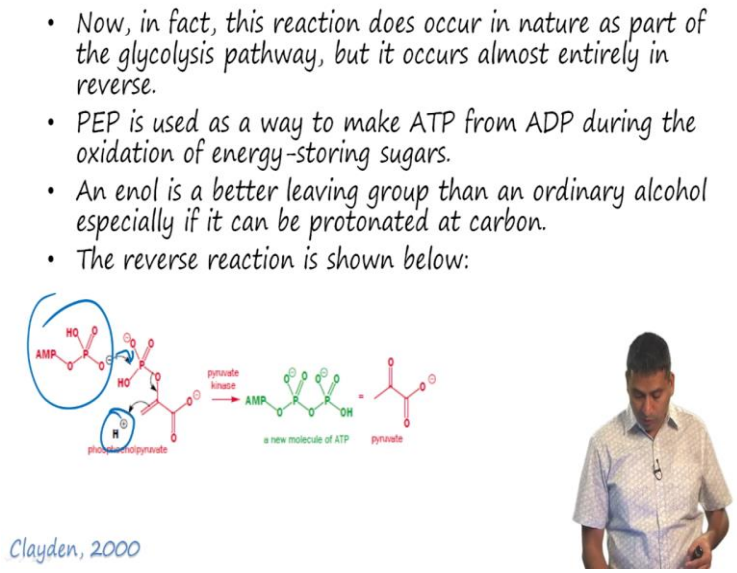
So here the phosphate attacks, so you can imagine that this is ADP

(Refer Slide Time: 23:18)



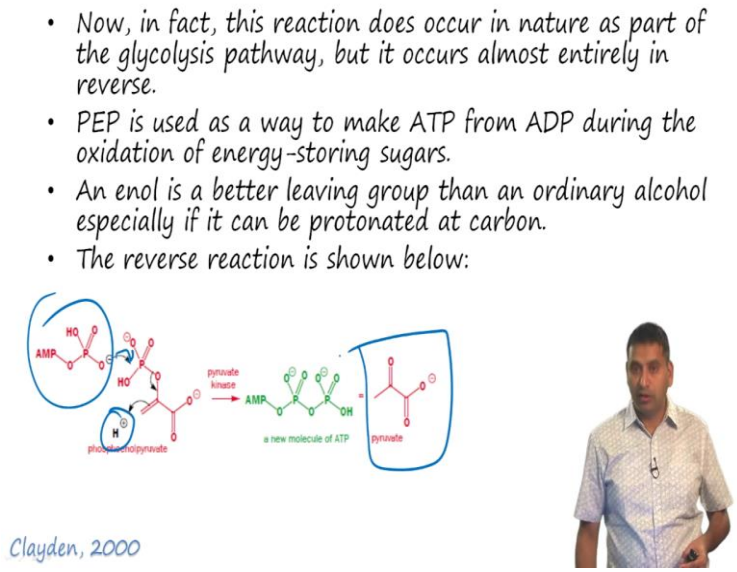
and the phosphate attacks and kicks out an enolate which is basically, or an enol which can pick up a proton

(Refer Slide Time: 23:26)



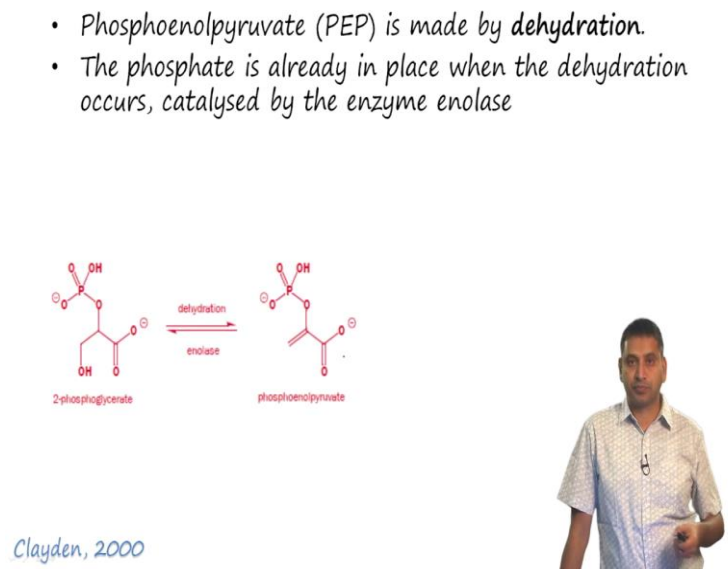
and give you pyruvate.

(Refer Slide Time: 23:28)



So here pyruvate is a very good leaving group because you have an opportunity for the double bond to pick up a proton.

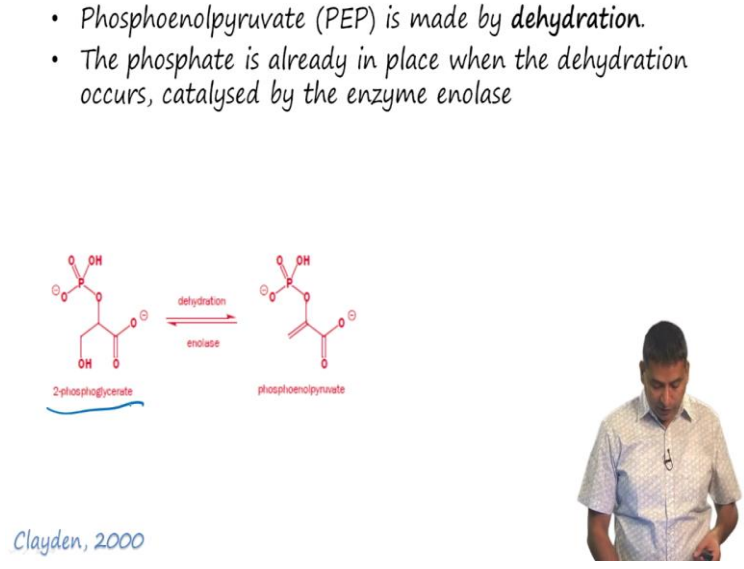
(Refer Slide Time: 23:35)



So phosphoenolpyruvate is made by dehydration, Ok. So the phosphate is already in place when the dehydration occurs.

And so this is enzyme, which is catalyzed by the enzyme called as enolase, Ok. So now let us look at these steps. So here is 2- phosphoglycerate

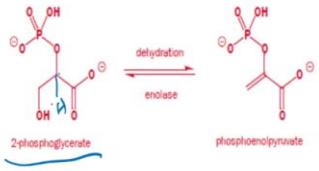
(Refer Slide Time: 23:55)



which is shown here. And if you can eliminate water from here

(Refer Slide Time: 24:01)

- Phosphoenolpyruvate (PEP) is made by **dehydration**.
- The phosphate is already in place when the dehydration occurs, catalysed by the enzyme enolase

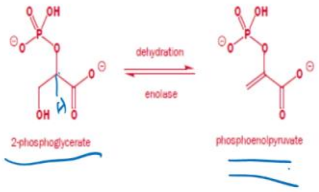


Clayden, 2000

then you end up with

(Refer Slide Time: 24:02)

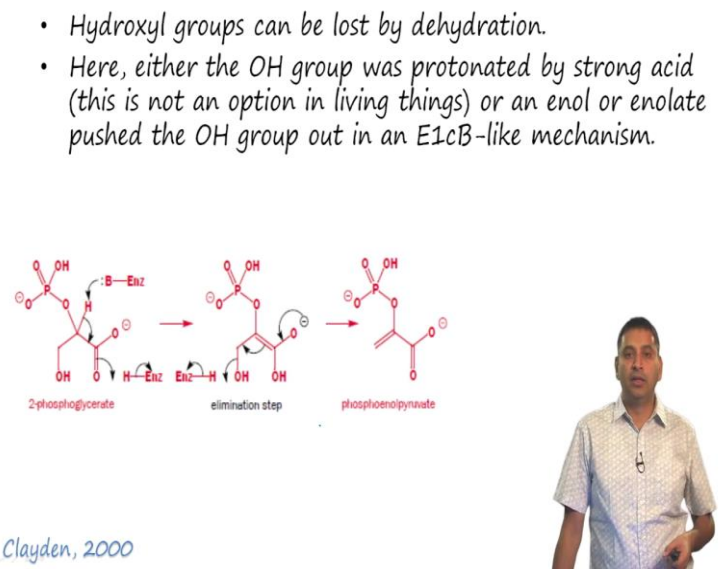
- Phosphoenolpyruvate (PEP) is made by **dehydration**.
- The phosphate is already in place when the dehydration occurs, catalysed by the enzyme enolase



Clayden, 2000

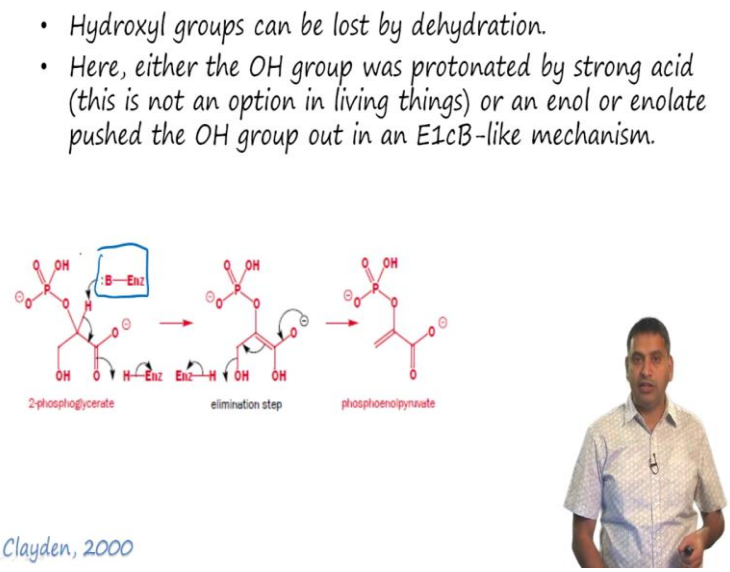
phosphoenolpyruvate.

(Refer Slide Time: 24:04)



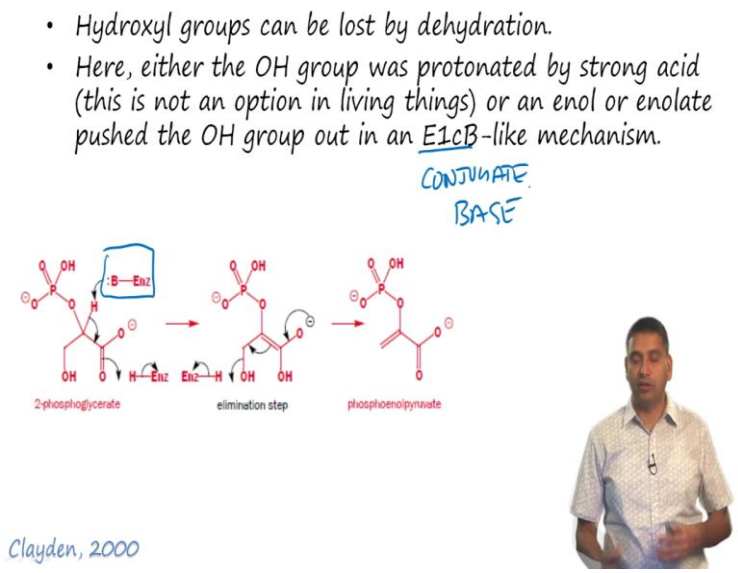
So the mechanism, by which this process occurs is by, is in the following manner. So the

(Refer Slide Time: 24:12)



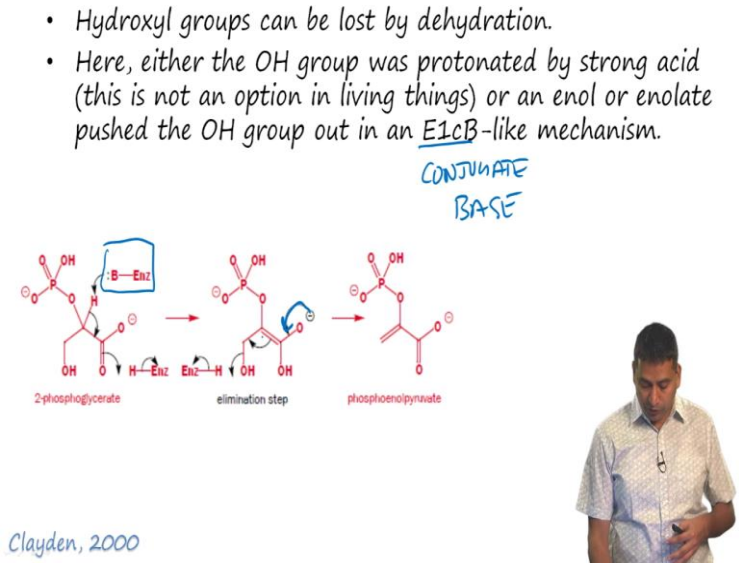
basic residue in the enzyme picks up this proton right next to the carboxylate and produces this enol and now this undergoes an elimination which is very much like E1cB like mechanism.

(Refer Slide Time: 24:32)



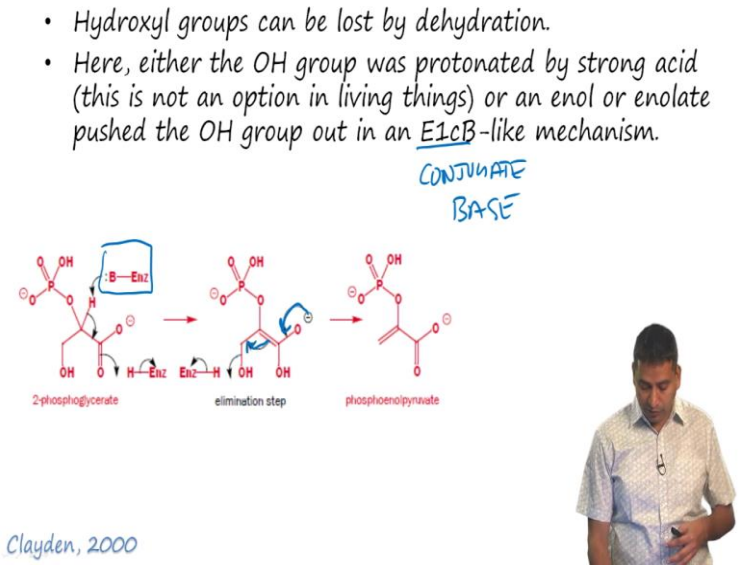
Again E1cB is basically E1 conjugate base mechanism which I am assuming people have looked at previously, and this elimination occurs in the following manner. So you have these electrons being pushed

(Refer Slide Time: 24:39)



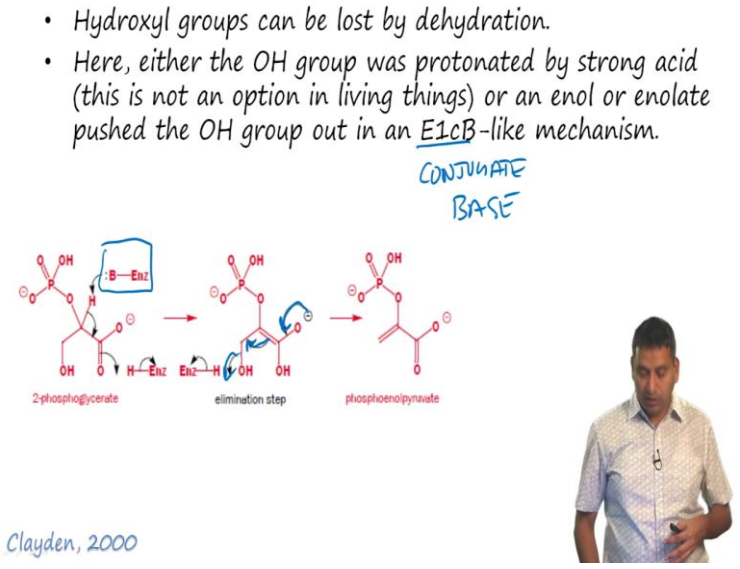
here, it then

(Refer Slide Time: 24:41)



kicks out the

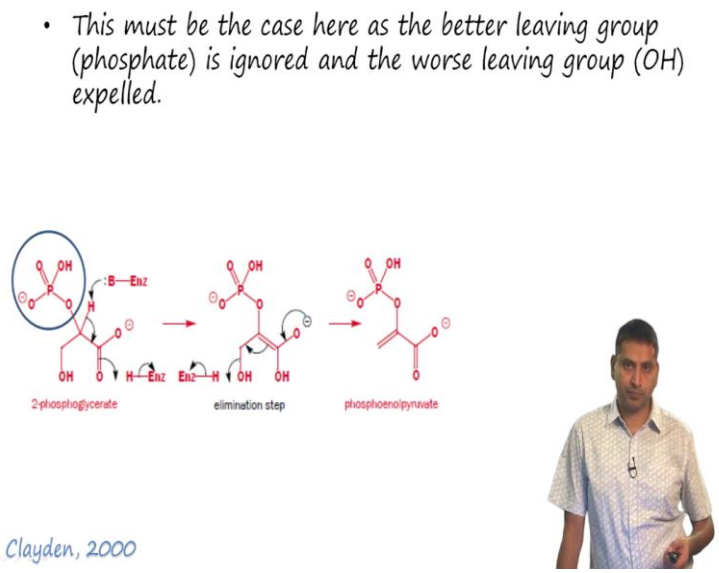
(Refer Slide Time: 24:42)



hydroxide ion to give you phosphoenolpyruvate.

So therefore phosphorylation of the enol does not occur but instead this phosphoenolpyruvate is synthesized by a process by which you form an enolate which then kicks out through an E 1cB mechanism a hydroxide ion to produce the phosphoenolpyruvate.

(Refer Slide Time: 25:03)

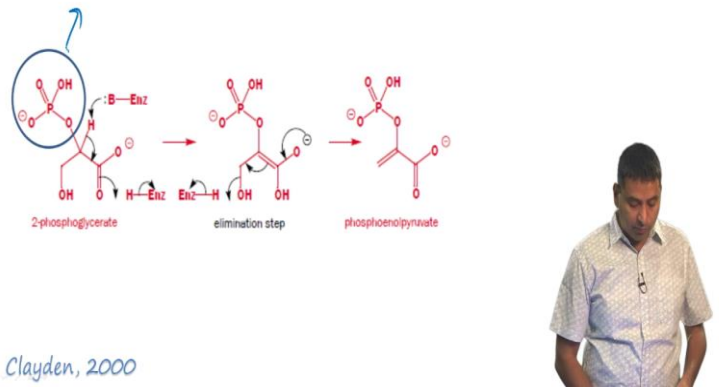


So this must be the case, here as the better leaving group phosphate is ignored, Ok and the worst leaving group hydroxide is expelled, Ok. So the way we understand this is expelled.

So if we were to produce this substrate by another mechanism then phosphate is actually the better leaving group and so because you are going through an E1cB mechanism this elimination occurs in the following manner.

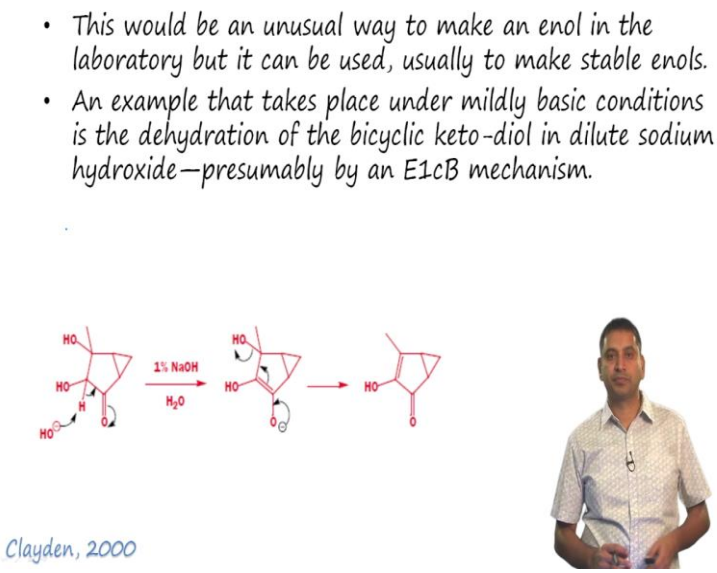
Otherwise what would happen is that this phosphate

(Refer Slide Time: 25:35)



would be eliminated and you will end up with the different product.

(Refer Slide Time: 25:40)

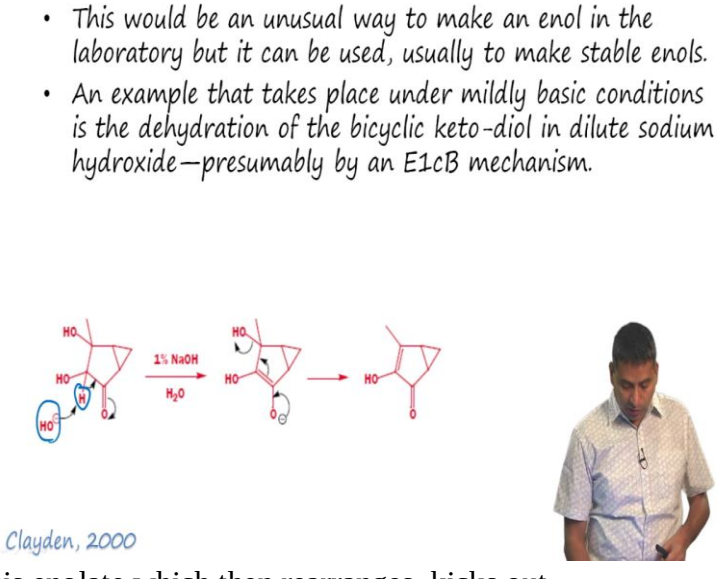


So this will be an unusual way of making an enol in the laboratory but it can be used usually to make stable enols.

So the example we can look at is this molecule here wherein you have, when you treat this with 1 percent sodium hydroxide you have an E1cB type mechanism that occurs.

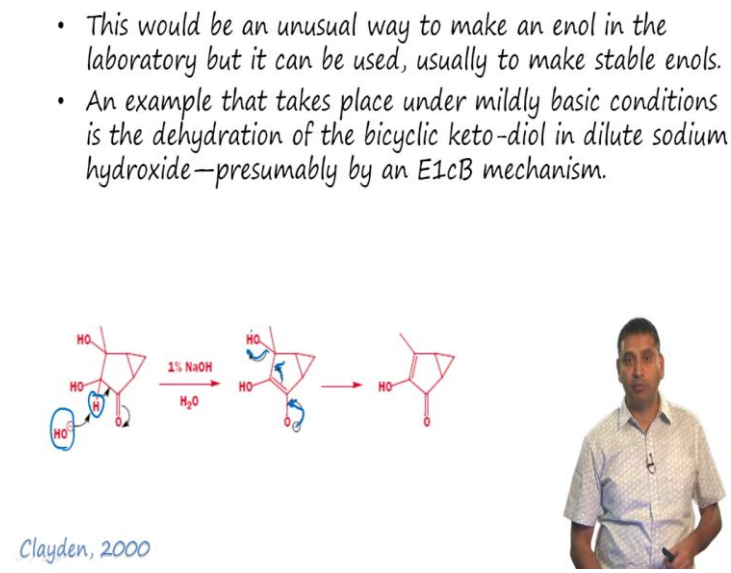
So first this proton is pulled out by hydroxide

(Refer Slide Time: 26:03)



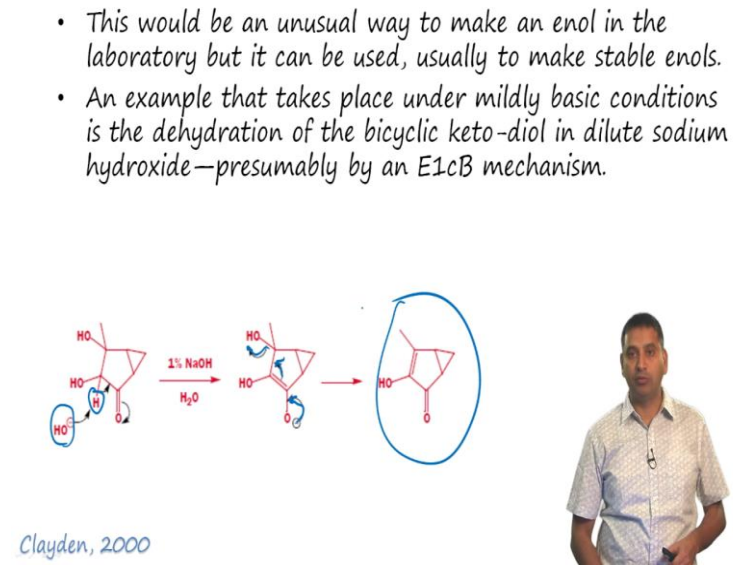
ion to generate this enolate which then rearranges, kicks out

(Refer Slide Time: 26:08)



OH minus in E1cB mechanism

(Refer Slide Time: 26:11)



to produce this product.