Organic Chemistry In Biology And Drug Development
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Lecture - 21
Synthetic Biology (Contd.)

In the last session, we have discussed how to produce the monoclonal antibodies by the hybridoma technology in connection with the generation of abzymes, which are basically antibody acting as enzymes. I ended up by saying that as organic chemist, what we can help in designing of the hapten.

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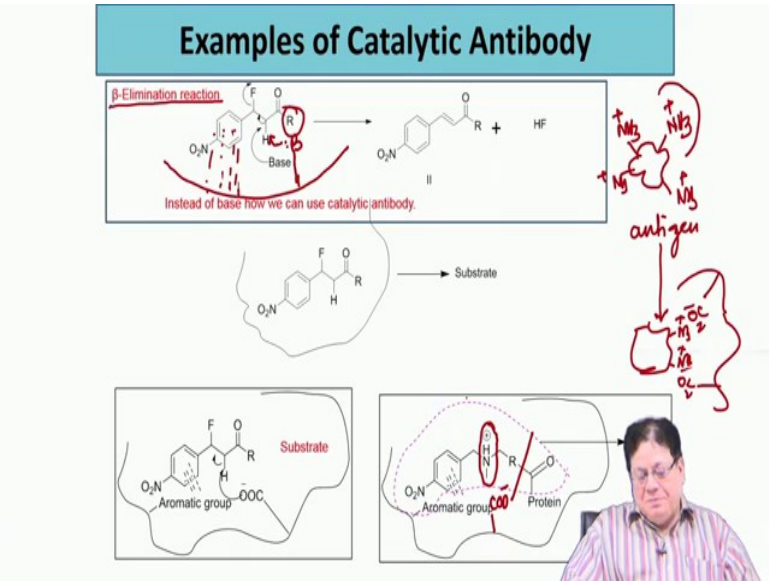

The hapten will be attached to the carrier molecule, and the hapten should have the epitope. Epitope is required in the hapten. What are the different strategies to come up which involved the hapten design? First strategy was really not the transition state analogue method. The simplest strategy (transition state analogue strategy) was that you know the transition state, make an analogue of transition state, attach it to the protein, and generate the monoclonal antibodies. But that was not the initial studies that were made.

This whole catalytic antibody was discovered in the western coast in the California particularly in an institute called Scripps Research Institute, where two scientists Richard Lerner and Peter Schultz developed this technology on catalytic antibody.

So, the first examples were based on producing the catalytic site in the antibody, because every enzyme has a catalytic site. This catalytic site contains amino acid residues that help in doing the reaction.. In the catalytic site of chymotrypsin, the catalytic triad is comprised of serine, histidine and aspartate.

So, if you can generate them artificially at the proper position, then you can make an antibody which will catalyze like chymotrypsin. But to generate three different amino acids at designed positions is really difficult. So, people went for actually producing only one active site amino acid, which is involved in catalysis,. So, the first technique that was used was basically generating the catalytic site which will catalyze the reaction. I think if I give an example, then it will be much clearer.

See there is a molecule: nitro phenyl moiety with fluorine at the benzylic carbon and then there is a β-hydrogen. And this hydrogen is quite acidic because there is a carbonyl group attached to it. We know that if I want to a make double bond here, I will definitely add a base. The mechanism for the reaction is that the base abstracts the hydrogen and the C-H bond electron pair goes here and the fluoride leaves. So, that is the simple mechanism. It is a β-elimination reaction

We have not specified R, because sometimes R may be some group which is very sensitive to base. So, you cannot use base in that case to induce this elimination, but we know that enzymes work at almost neutral pH, so that is another advantage of catalytic antibody; instead of base (which can do this elimination), can we can generate a catalytic antibody which will also do this elimination process? That will be very useful because that will be under very mild condition. Of course, this is an example which is nothing but they wanted to have a proof of concept that yes this is possible. You can generate the particular amino acid responsible for catalysis at the catalytic site which is the active site.

If I have the antigen which is invading our system, suppose that antigen has lot of amino acids like lysine.. Its surface will be full of lysines which will be positively charged at physiological pH. Now, if an antibody is generated against it, then we can definitely assume that the antibody must be such that it must be able to recognize this antigen. Thus the antibody must be such that it has got lot of carboxylate groups. Because only then there will be an interaction with the NH_3 plus, otherwise there will be possibly no recognition between the antigen and the antibody.

Thus for fruitful recognition, if the antigen has lot of positive charge on the surface, the antibody will have lot of negative charge on its surface when it binds. Similarly, if the antigen has lot of aromatic rings, then the antibody will also have aromatic rings because that will give you π-π stacking interactions. So, these are some very simple things with which you can predict the probable structure of the antibody depending on the structure of the antigen.

Now, in this case remember the antigen what you are using is basically your reaction substrate. So basically I want to generate an antibody which is a protein; instead of the base, the protein has a base at that position where the hydrogen is residing.

If the protein has a base which is generated here by manipulation, then the same reaction will take place, because what you need is a base in proximity with the hydrogen. This molecule binds to this antibody; that is also very important; some binding parameter has to be there for which it binds and the base is right in front of the hydrogen. So, now the base will abstract the hydrogen and the elimination process will take place.

Now, that means, what you have to do? When you design the hapten, you have to think that how can I generate a basic center at the catalytic site. I already told you that if the antibody is positively charged, then you get a negative carboxylate in the antibody. Now, here you want a basic group. What is a basic group in a protein at biological pH? Sometimes we think that the basic groups are arginine, lysine etc.

But the problem with this concept is that at the biological pH, lysine is present as a NH_3 plus; its lone pair is no longer available; similarly arginine will be also present as the positively charged conjugate acid. So, they are not bases in the biological system (at the pH 7.2). So, what are the bases then in biological system? A base is something which has got excess electron, either the lone pair or it could be negatively charged like OH minus.

Which amino acid residue exists as the negative charge in biological conditions? The amino acids that have carboxylic side chain; because carboxylic acid at biological pH will exist as carboxylate; like glutamate or aspartate present as the anion; so that is your source of base. So, if that means I have to generate a carboxylate at the active site, how can I do that? See here it is written; if I have a hapten like this where I put a NHMe plus here, that means, the hapten has a plus charge somewhere in the middle (where there was the hydrogen attached).

So, I have a positive charge here. I keep this aromatic ring intact; other things more or less intact; only I have to attach the large carrier protein. So, up to this point is your hapten and then you attach it to the protein; this is the hapten. In the hapten design, you will notice that this part is not changed. That is because this part will make another aromatic amino acid here which will assist recognition; thus this nitro phenyl group has a stabilizing interaction by π-stacking. And this NHMe plus will generate a carboxylate here in order to have this antigen-antibody stabilizing interaction. Exactly that was done.

So, this molecule was injected to mice, and the monoclone antibody was isolated. And each had an aromatic group here which stabilizes the nitro phenyl moiety; and it had a carboxylic group right where you wanted, and this then underwent elimination reaction as desired. So, this is one of the first examples of catalytic antibody, but again I repeat, that this is not the transition state analogue approach, this is an approach which directs the generation of the catalytic residue in the antibody.

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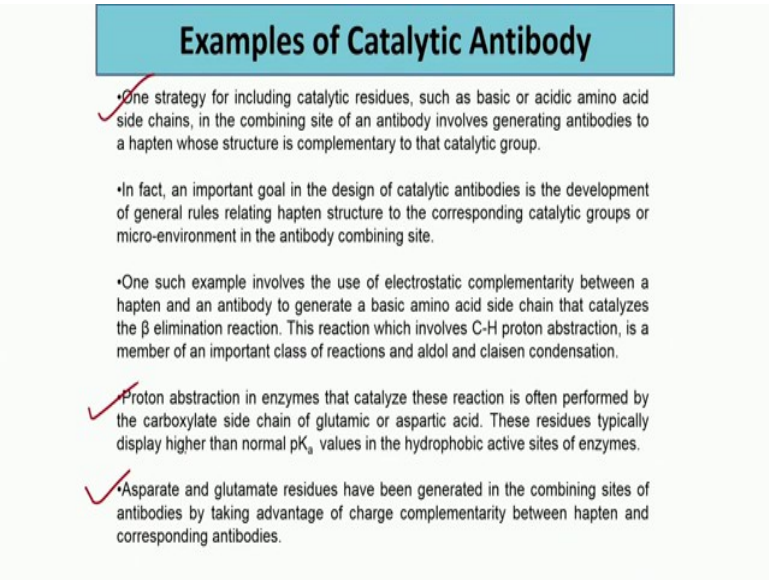

Now, let us go to other examples. I just told you that this is the first strategy which involves the generation of catalytic residues. I have given you generation of a carboxylate. Similarly if in some reaction, you want generation of a positive charge (something like NH_3 plus), then you have to use a carboxylate in the hapten, which is just the reverse way.

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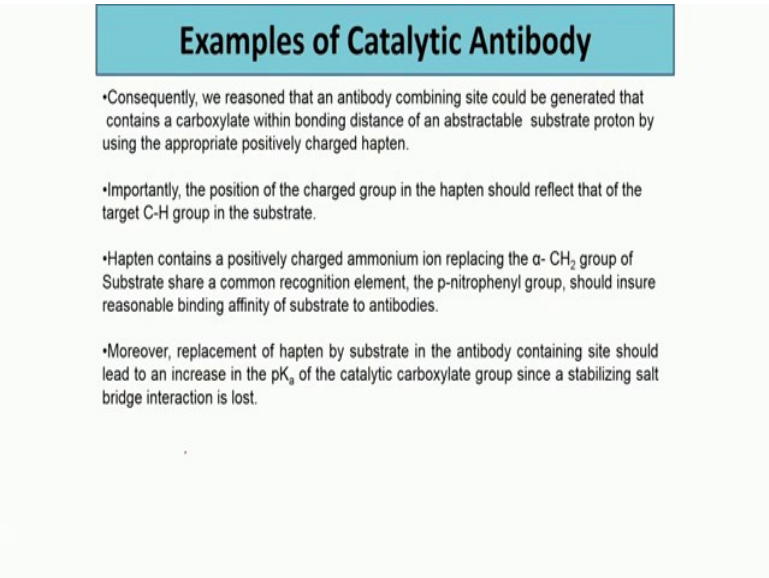

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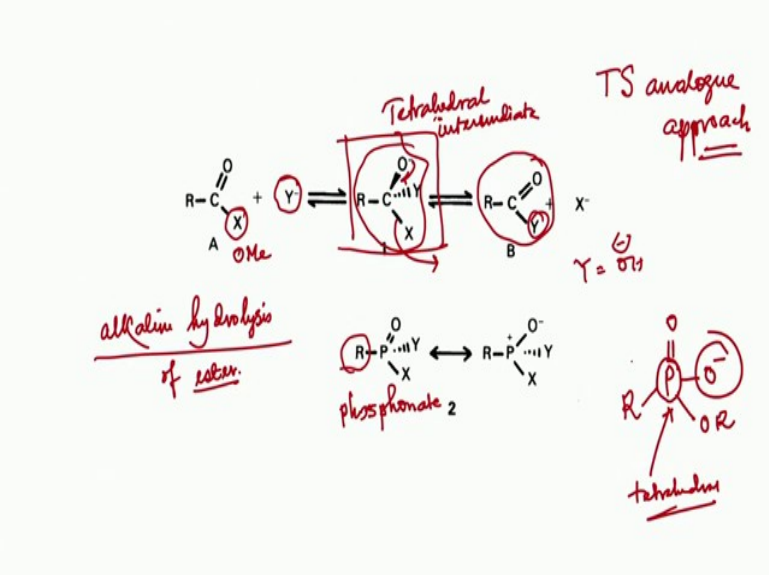

The next one is the transition state analogue approach. And the first reaction that was tried was the hydrolysis of an ester. Here this is X; suppose this X is OMe; we are considering alkaline hydrolysis of an ester. Now, this is also called saponification (alkaline ester hydrolysis). Now, the mechanism of ester hydrolysis by base is discussed here. Suppose this is your base. So, the base first attacks and forms what is called a tetrahedral intermediate; this is a tetrahedral intermediate. And this is your rate determining step, this attack by the

nucleophile on to the carbonyl carbon. It is a bimolecular reaction, two molecules are involved to form the intermediate via the transition state.

And the next step is this O minus comes back and this leaving group leaves. So, you get the hydrolyzed product. This is Y, suppose the Y is OH minus then you get the carboxylic acid. That means, in hydrolysis of an acyl system, an ester goes through a tetrahedral intermediate via a transition state.

The intermediate is the one which is closest to the transition state. And so when I do the hapten design, I can take the intermediate and see which molecule resembles this intermediate; the intermediate is stable, because if it is not stable, then nobody could isolate this. You have to understand that once it becomes O minus, it is very transitory; this comes back and X leaves. So, you cannot have this taken in a test tube and seal it and that will remain forever; that is not true, this is also transitory.

So, what you need is a stable molecule which looks like this; that means you should have a molecule which is because this intermediate is tetrahedral; secondly, it should have a negative charge on the outward atoms. So, what you need is your R group; I said that do not disturb the R group because depending on the R group, your binding parameters are decided. What you do is take a phosphonate.

See basically in phosphonate, phosphorus is directly attached to a carbon . P is attached to O (double bonded), and O minus, OR and R (single bonded). This is a stable molecule. Now, look at the structure of the tetrahedral intermediate and the phosphonate; instead of carbon, what you have is a phosphorous, but this is tetrahedral. And this has also got a negative charge; like the carbon O minus, you have phosphorous O minus. So, instead of carbon, you have just a phosphorous, which is just slightly bulkier then carbon; that is the only difference.

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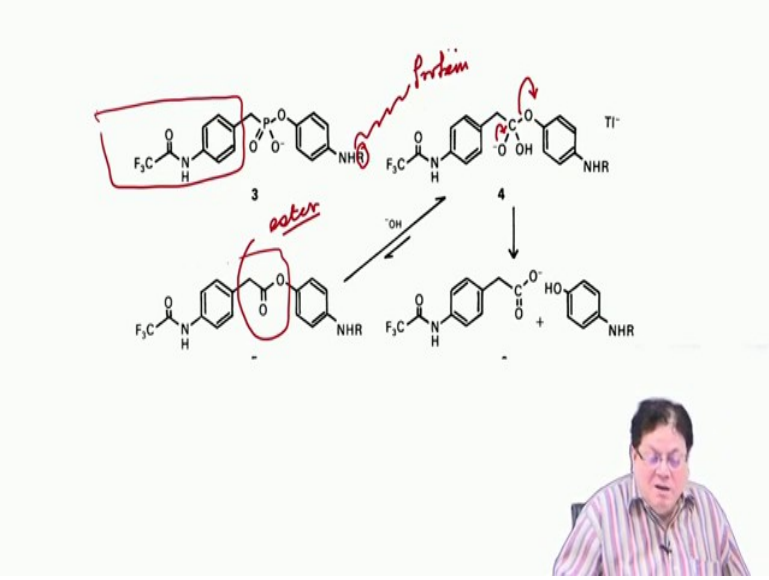

Let us take an example. This is A, where this is the ester. You want to hydrolyze it, via normal saponification using OH minus. So, this is the tetrahedral intermediate, and in this tetrahedral intermediate, it comes here, and this group leaves; this groups leaves here. So, you get the carboxylic acid and the corresponding phenol.

In this case, the phenol is the alcohol part; so that is the mechanism. This is your intermediate. So, what will be your hapten? The hapten is this part which you do not change; again I repeat that this part remains the same. Now instead of carbon, you take a phosphonate; P double bond O, O minus and the other part remaining the same. Only thing is that through this R, you attach it to a linker and then the protein..

So, this is the hapten, it resembles perfectly the intermediate and the intermediate is close to the transition state. So, if you can generate an antibody which recognizes this ensemble, then that is going to catalyze the hydrolysis of this compound; so that is very simple, and they have done.

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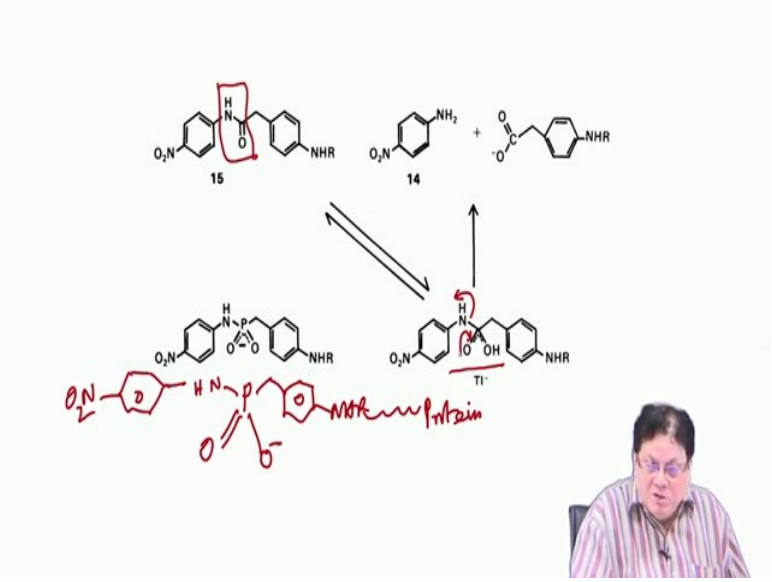

Suppose you want to hydrolyze an amide; like all those proteases which hydrolyze the amide bond. You can do it with alkali. So, if you add alkali, again you have that tetrahedral intermediate, and then the tetrahedral intermediate collapses, and this goes out, and you get the carboxylate and the nitro amine. You want a catalytic antibody to catalyze this reaction. So, now you generate the hapten. Here instead of carbon, you have nitrogen.

So, this is nitrogen, adjacent to nitrogen is this phosphorous. This is called phosphoramidite. So, this is very simple; the reactions are not very difficult. The reactions basically involve synthesis of catalytic antibodies for the hydrolysis of amide or for the hydrolysis of ester. They have done also hydrolysis of carbonate.

You might say that sir these reactions are quite easy, but as I said in the actual case, you might have certain groups which react under these conditions. This is very mild when you use the catalytic antibody. And the other thing is that, since these are enzyme like reactions, the turnover number will also be very high; unlike the hydrolysis under the basic conditions (saponification), the turn over number is also very high.

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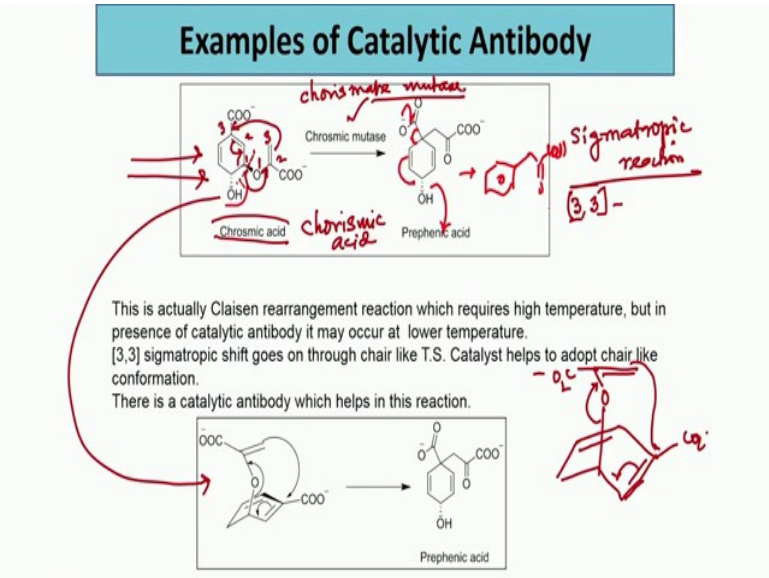

Now, we come to a more difficult reactions. You know that some of the amino acids are essential, some are non-essential amino acids. Essential amino acids mean you have to give them from outside. Some of the microorganisms, can make one particular amino acid like phenylalanine which we cannot make; phenylalanine has to come from outside.

Now, the biosynthesis of phenylalanine is little complex, but we have taken only some intermediate steps where phenylalanine, biosynthesis is involved. This is a molecule which is called chorismic acid. There is some biosynthetic path way through which this molecule is generated.

An enzyme which plays a major role is this biosynthesis is chrosmic mutase. But actually, it is chorismate, because all these acids are actually present in the carboxylate form; that is how you always have glutamate, aspartate. So, this will be chorismate mutase; it is known as mutase because basically it is an isomerization reaction where the skeleton changes; isomerization means molecular formula of these is same as that. So, it must be a kind of a rearrangement reaction.

So, that isomerization reaction is done by chorismate mutase; mutase means where the skeleton changes. The skeleton looks entirely different from what was there. Now, if you want to do this reaction chemically, you can do it;but you have to heat it at a very high

temperature. This is an example of sigmatropic reaction. Specifically this is called a [3,3] sigmatropic reaction. In these, both ends of the relocating σ bond migrate three atoms.

If you look at all these, the sigma bond that is broken is this one; and then this will have the numbering as shown. So, this becomes 3 and this is also 1, and that is 2 and that is 3. So, ultimately the 3 and 3 carbons are combining with each other, so that is why called this is called a [3,3] sigmatropic shift.

This is not the actual geometry through which the reaction takes place. The actual geometry is shown here. It takes the shape of something like an inverted boat. And then you have this oxygen, you have that carbon, then the double bond, and this CO_2 minus, and here is double bond.

During the reaction, the molecule takes up this geometry. You may argue that the carbon atoms present the 3, 3 positions are quite far apart, how are they reacting with each other? But in this structure (that has been shown), you can there is no such problem. So, this is the O then that double bond and this is the CO_2 minus, and here you have a CO_2 minus. So, when the reaction takes place, the arrow goes like this, you have a transition state which will looks like a chair;

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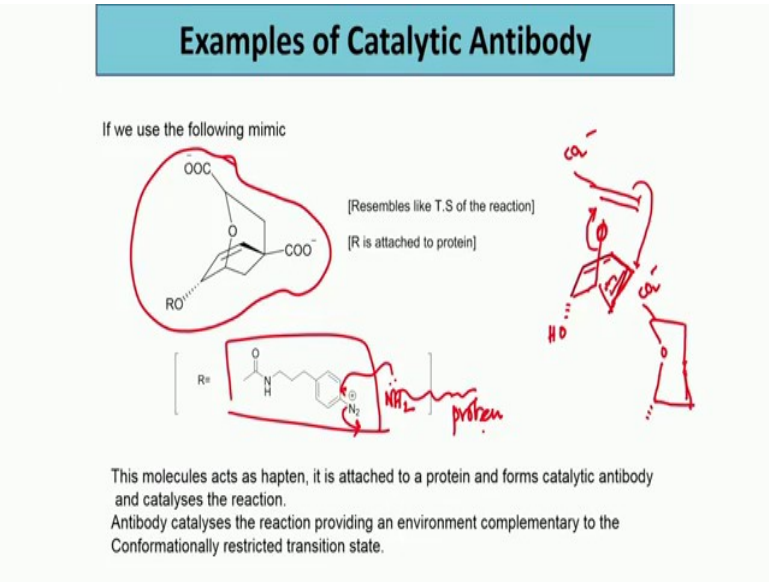

So, this is the your this is the oxygen, this is the double bond, this is the CO_2 minus, and earlier there was a double bond here and a double bond there, and there was OH here.

So, now, the reaction goes by this pathway as shown. So, what you have is oxygen, then you have this which id close to a chair. If you look at this position, more or less like a chair. So, it is a chair like transition state which is involved in this process, and that is true for all [3,3] sigmatropic reactions; they go via chair like transition state. Examples are Cope rearrangement, Claisen rearrangement etc.

Now, earlier people used to think that pericyclic reactions occur either by a heat or by light. People used to believe that pericyclic reactions cannot be catalyzed. They are unaffected by catalysts. But here is an enzyme chorismate mutase which does the rearrangement very fast at room temperature; you do not have to heat it. So, that actually broke the myth that pericyclic reactions can be catalyzed. How the enzymes are catalyzing it? Basically the enzyme helps the molecule to adopt a conformation through which the reaction takes place.

The enzyme helps the molecule to bind, adopt a conformation through which the reaction takes place. So, if you want to make a catalytic antibody to have this reaction catalyzed, what you need to do is make a transition state like molecule which is stable like this. See this is the transition state which we are talking about there is a OH here earlier; remember there is a OH here in chorismic acid, so via that OH, you attach it to the to the linker. The linker was a diazo compound and through the diazo compound, you attach the protein; because diazonium salts are prone to attack and the nitrogen leaves.

So, by that you can attach your protein part. So, basically what you have done? You have made a molecule which looks like the transition state, but this is stable; you synthesize this molecule and via this OH, you attach it to the carrier protein; now you generate catalytic antibody and that antibody has been very successful; that antibody catalyzed this chorismate mutase reaction. Chorismic acid going to prephenic acid. This is the intermediate for the phenylalanine synthesis; so this is a very critical step. Since this is an essential amino acid, so this has to come from outside. The question is how the plants or the microorganisms make phenylalanine? So, this is the route.

So, chorismic acid rearranges to the prephenic acid, then there is a decarboxylative dehydration; but here OH is a bad leaving group, so is converted to a phosphate, so that leaves and that gives you a compound which contains this CO CO_2H (α-keto acids). When we will read some more coenzyme chemistry, we will see that these α-keto acids can be

converted to amino acids. So that is the story of phenylalanine and that has come because of this catalytic antibody.

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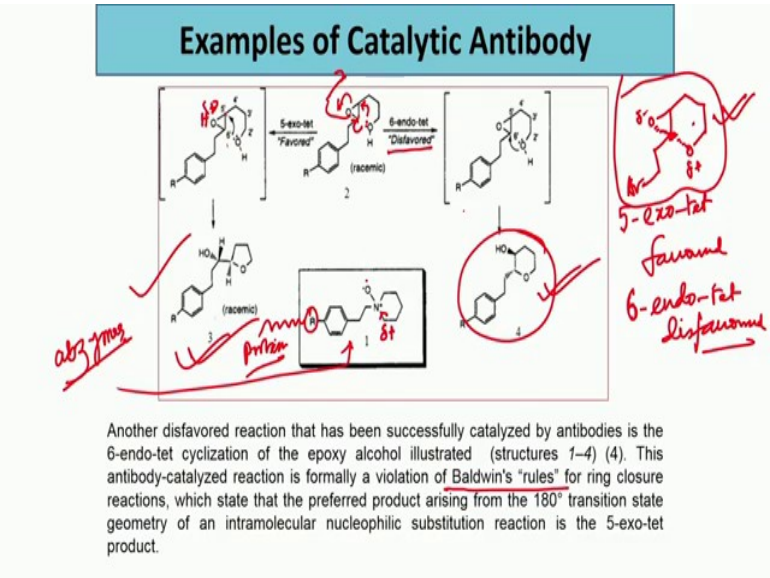

The last reaction that I am going to discuss is involving those reactions which are disfavored reactions; which do not happen under standard organic reaction conditions. Here you can force the reaction by catalytic antibody to follow a disfavored path way. How does this happenLet us take this example, this is a phenyl with some substituent, then CH_2CH_2 and an epoxide and then the epoxide is connected ultimately to a hydroxyl group.

Now, if you add some acid, this the epoxide will be protonated and as soon as its protonated, since epoxides are strained molecule, they will open up. Their opening is assisted by this adjacent nucleophile which is OH. So, now you have two modes of reopening; where by the OH can attack either of the two carbons involved in the three membered epoxide ring.

One is called 5-exo-tet. What is tet? Tet means you are attacking at a tetrahedral carbon, because these are tetrahedral carbon sp^3 hybridized, so tet. Why it is called 5-exo? That means, when it is attacking that one, what you are making is a 5 member ring, so that is 5. And your whole epoxide is outside the ring that is being formed, so that is why that is called exo; so this is 5-exo-tet.

The other possibility where the lone pair attacks this carbon, now this whole bond containing the epoxide becomes a part of the ring, so this will be called endo, but again you are attacking to a tetrahedral carbon, but you are making a 6 member ring, so this is called 6-endo-tet.

There are some rules called Baldwin's rules (proposed by Sir Jack E. Baldwin) which govern these cyclization reactions. If you do this reaction, you will see that this is the product which is formed because Baldwin's rules say that 5-exo-tet is favored and the 6-endo-tet is disfavored.

So, in the in the test tube, if you do this reaction and the chemical reagent used is acid, like some Lewis acid, you will get this product as the major product; but if your target is to make the other possible compound, then the question is how to make a disfavored reaction favored? So, what was done is that when this OH attacking in the disfavored reaction which is the endo,

part of this C-O bond is now broken; so this will be δ- and this oxygen will be δ+; maybe I can draw the transition state; so that is δ- and then what you have is oxygen; these are half bonds (these are not formed fully) and this is the aromatic ring and that will be δ+. Now if you can mimic this with a molecule then that will be your hapten, and with that hapten, if you can generate a catalytic antibody and give it to this system, then that will do this reaction (6-endo-tet).

So finally they come out with a molecule which is an N-oxide molecule. Now, look at this molecule and try to check here, see what you need is a carbon here which may not be carbon, you can have different atoms, but it should be attached to a kind of negatively charged oxygen. Instead of the carbon you a have nitrogen here and you have O minus here.

But what you needed is a δ+ center at the next carbon; and you want a minus on oxygen. Now, because this nitrogen is positively charged N-oxide; so due to inductive effect, this will generate a δ+ on the adjacent carbon as shown. So, now if you look at this one and that one, they are they resemble each other. See this is tetrahedral, that is tetrahedral;, this has got a negative charge, this is the negative charge and this is the positive charge and this positive charge is generated by inductive effect and this R is utilized to attach the protein.

So, now if you can generate a catalytic antibody against this, then that will act as a catalyst do a disfavored reaction and ultimately you get this product. In fact, this was done and this was a

very classic example and it showed the power of catalytic antibody to do unfavored reactions. There are many more examples of unfavored reactions by using catalytic antibody approach. We do not know any other approach by which you can get a disfavored reaction into a favored process. So, I think that ends this aspect of synthetic biology that involves the abzyme chemistry or the catalytic antibody chemistry.

Thank you.