Organic Chemistry In Biology And Drug Development

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Lecture - 19
Synthetic Biology

Welcome back, we will now talk about something which possibly deals with a subject that is about 30 years old. Actually this whole area comes under the domain of called Synthetic Biology.

Synthetic chemistry like synthetic organic chemistry is basically making new entities, new molecules; either you make new molecules or it could be some natural products which is existing in the plant or other microorganisms. Synthetic material can be like our synthetic textile (polymer based) and then you have the natural textile which might be made of cotton. A question was raised that can we synthesize typical biological entities? Can we make them synthetically or artificially in the lab, and make them do the reactions or execute the functions, which proteins or other biomolecules are doing? So, is it possible to design our own catalytic systems, which are like enzymes? So, that is one domain of synthetic biology where we are trying to make synthetic enzymes in order to catalyze a particular reaction.

Remember nature has given us these proteins which act as enzymes. How many enzymes are there? It could be a huge number that is true. What type of reactions do they catalyze? That also depends on the nature of the enzyme, but I have given you six classes of enzymes: oxidoreductase, transferase, hydrolase, lyase, isomerase and finally, ligase. Now, when you isolate enzymes from natural sources (since enzymes cannot be isolated from unnatural sources), these enzymes are actually tailor made by nature to catalyze a particular reaction. Organic chemists are always looking for catalysts to catalyze their reactions. Now, number of reported organic reactions is huge; there are different types of reactions; but the number of biochemical reactions may be restricted. Actually it is restricted because it depends on the number of enzymes that are available.

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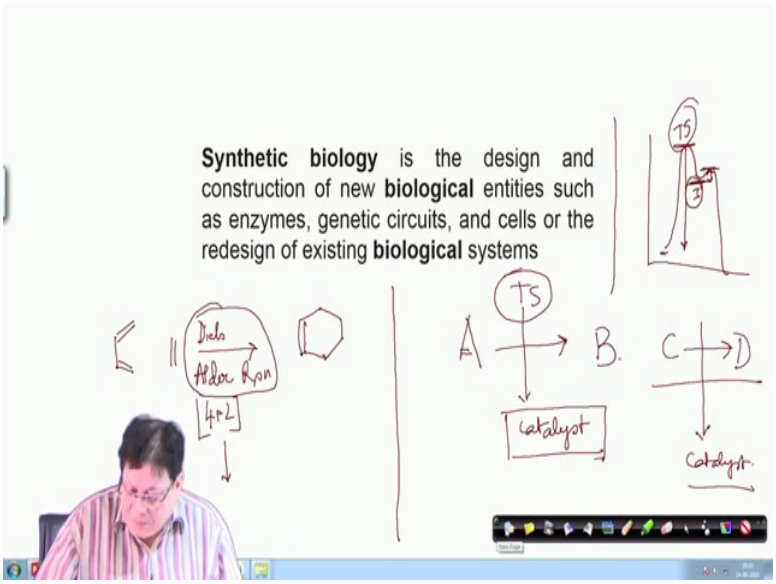

I give an example; there is a reaction which is very famous; it is called the Diels Alder reaction. So, what is made here? You make a cyclic compound; the double bonds rearrange and you get cyclohexene, this is also called a [4 + 2] cyclo-addition reaction.

In order to have the Diels Alder reaction, you have to take these two systems: one is butadiene and another is ethylene. You have to take them in a flask, add the solvent and then heat it; unless you heat it, this reaction will not go. Although there are some Diels Alder reactions which proceeds at lower temperature, but a majority of them need refluxing in solvent at high temperature.

Enzyme reactions are happening at what temperature? They happen at the biological temperature. What is a biological temperature? Biological temperature in a human system is about 37 °C. So, in our body, at about 37 °C, reactions are happening. For enzymes, heating has a negative effect on its activity. If you heat the enzyme, what happens? The enzymes are actually folded in a three dimensional network that we showed; which is called the tertiary structure. So, as we heat the enzyme, the conformation that is required to catalyze the reaction changes and if that changes, then the enzymes will ultimately loose the activity.

So, enzymes can catalyze reactions at room temperature or the biological temperature (30 to 37 °C). So, can we design an enzyme for Diels Alder reaction? If we can design an enzyme and then synthesize that; then we will have this reaction which will be catalyzed by the

enzyme. Why synthetic biology is required? Because the question is whether there is any enzyme which does this Diels Alder reaction or not.

Today, there are few enzymes available to carry out the Diels Alder reaction, but may be 20 years back, people thought that this pericyclic reaction could not be catalyzed by enzymes. So, they were after making this type of biological systems; taking the help of biology. I will show you how you can make those synthetic or artificial enzyme like molecules, as you cannot call them enzymes because enzymes are natural. So, they are enzyme like molecules.

Take a general case. I want to have a reaction where there is substrate A, I want to convert it into the product B. First, I will search whether there is any enzyme which does this reaction. Suppose there is no enzyme available to do this reaction. Actually many of the organic reactions are not catalyzed by enzymes that we normally do in the laboratory.

On the other hand, there are different types of enzymes that are used in synthetic organic chemistry to carry out certain transformations. Now, suppose for this A to B conversion, there is no enzyme available; if enzymes would have been available then people would not have gone after making new enzymes to catalyze this reaction. Because enzymes have been designed and ultimately made through evolution by nature. Nature made these enzymes perfect for certain reactions or selective for certain reactions over millions and millions of years.

If you think that nature is a craftsman or craftswoman, where he or she tried all these things and ultimately came out with the best possible protein which can act as the enzyme. For the same type of enzyme, it will be very difficult to compete with the natural system. So, usually the synthetic biology people look for reactions for which no enzyme is known.

Suppose for this transformation from A to B, there is no enzyme that is known; but I want an enzyme like catalyst. There are different catalysts in organic chemistry that are used like transition metal based catalyst, wilkinson's catalyst, raney nickel, palladium on charcoal; all these are catalysts; some are homogeneous some are heterogeneous. We are not talking about those types of catalysts; we are talking about a catalyst whose mechanism of reaction is very similar to the enzyme. So, that was the desire of organic chemist.

If that strategy becomes successful, and you are able to catalyze the reaction of A to B, then by following the same strategy, you can catalyze a reaction from C to D. So, for any reaction

which is known to occur in synthetic organic chemistry, virtually you can design an enzyme like catalyst. So, that is the whole scenario; although we are far from that position where we have catalyst for each reaction; still there are some difficulties. I will tell you what are the difficulties; but the idea is to have a catalyst catalyze a reaction where no enzyme is possibly known.

And if your strategy is correct, if your principle is correct, then in theory, you can catalyze any reaction. Of course, there is one question that will be asked; what is the mechanism of that organic reaction? Unless you know the mechanism, you will not be able to design an artificial enzyme like catalyst. Why the mechanism is required?

Because if you know the mechanism, then only you know what is the transition state for the reaction. In order to know the transition state, you need to know the mechanism of the reaction. Suppose for the reactions S_N2 and S_N1, the transition state for both the reactions are different. So, unless you know the mechanism, you will not know the transition state; so, that is why the mechanism has to be established.

Today with the advancement of spectroscopic techniques, many of the mechanisms for organic reactions have been revealed. So, mechanism is not a much bigger problem these days; we know the mechanism. So, if we know the mechanism, we we can roughly draw the transition state. It is also very difficult to pin-point the structure of the transition state.

Remember what is the transition state? Where bond formation and bond breakage are almost half way through; they are not complete. Some may be three fourth through; that means, the bond breakage is more and bond formation is less. So, it is really very difficult to know the actual structure of the transition state. But one may consider the intermediate involved in the reaction. I told you last time that according to Hammond's postulate; transition state structure resembles mostly the intermediate for that reaction and that is simply because the energy profile diagram is something like this.

So, this is your intermediate and this is your transition state. Now there are two transition states for this reaction; however, always we look for the slowest step of the reaction which determines the rate of a reaction. So, this has got an activation energy which is much higher than the activation energy for the second step and this is the intermediate. Now; that means, we will be interested to know what is the structure of this transition state and this is closest in energy with the intermediate and not with the substrate or the product.

You know that some intermediates can be isolated, their existence can be proven, their structures can be predicted. So, if you know the structure of the intermediate; you can assume that this will have a very similar structure as that of the intermediate.

Now, how to develop an enzyme like catalyst? The principle is very simple that you know that in the enzyme catalysis, substrate binds to the enzyme, then it goes to the transition state; the transition state is also bound to the enzyme and then it goes to the product; the product was initially bound to the enzyme then it is released. Now, out of these three species (substrate, transition state and product), the enzyme offers varying order of stability to the substrate, transition state and the product.

The enzyme offers least stability to the product because product has to be released very quickly so that new enzyme molecules are made. This transition state is the one which is stabilized most because that will lower the activation energy. So, out of these three species, transition state will be the most stabilized one by the enzyme. That means, the transition state has a structure which is geometrically and electronically most complementary to the structure of the active site in the enzyme.

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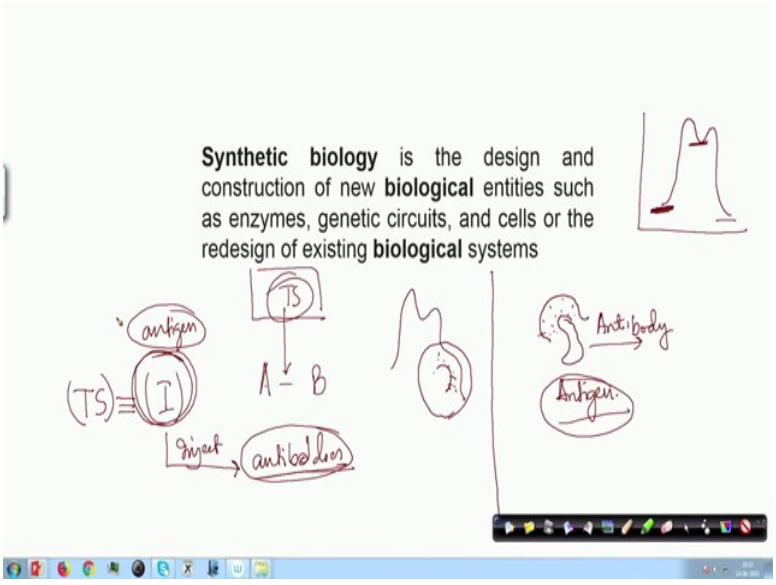

So, what you need is that you should make a protein like molecule and that should have some site which is electronically and geometrically complementary to the transition state.

So, suppose this is the active site of the transition state, the substrate goes and binds then it becomes the transition state and it is stabilized by different types of weak interaction. Now the question is how to create it? So, the problem boils down to creation of the active site in the enzyme.

Now, what is the technique that people have adopted? We know that if a foreign particle or foreign organism enters into our body, something is generated in the blood stream and that is what are called antibody. So, when some molecule comes and invades and it is in the blood circulation; then what happens? Some molecules are made by our body system; it immediately recognizes that some foreign substance has entered in my body; so there is something what is called immune response.

So, our immune response takes some time and then it immediately makes the antibodies. What are these antibodies? The antibodies are the ones which recognize the invading molecule; that means, this antibody has a structure which is complementary to the invading molecule structure; otherwise it could not have recognized the invading molecules.

Remember the invading molecule is called the antigen. Now, if you can generate antibody against this, then the antibody will have a geometry which will definitely look something like this in order to have the complementarity against the antigen.

So, basically what I said? That if some molecule enters into the body then there is an immune response and some proteins which are antibodies (also called immunoglobulins) are generated which have complementary structure to the antigen. Now, suppose I make an antigen which looks like the transition state of a reaction.

So, my antigen is basically the transition state, but remember the transition states are very unstable and transitory in nature. So, better this is approximated to the intermediate structure because intermediate lies in the energy minima. If you go to the physical chemistry side now; so what are isolable here? The substrate, intermediate and the product are isolable.

This is the intermediate; that means, intermediate has stability. So, you can make something which looks like the intermediate. So, now, suppose you have the intermediate in hand. So, what should I do? I should inject the intermediate into the mice. I will not inject into a human body, since that will not be allowed; ethically not correct.

So, you inject into the mice and then what will happen? I will expect that there will be immune response in the mice and if there is immune response, then antibodies will be generated. And if antibodies are generated, then these antibodies will recognize this intermediate because this is your antigen now. So, that molecule will be recognized by the antibodies and then that will bind to this intermediate.

Then there are complex biological processes (immunological processes) which ultimately destroys the antigen. So, now you come back again, I want to have a catalyst to catalyze the reaction A to B. I know the mechanism of this reaction; this mechanism has an intermediate produced in between the substrate and the product; I know the structure of the intermediate.

. If I take the intermediate (which has some stability) and inject it into the mice then what should happen? What will I expect? I will expect that antibodies will be generated because the intermediate molecule is a foreign particle. And if I isolate these antibodies; these antibodies are the ones which will recognize the antigen; in this case the antigen is the intermediate.

So, then these antibodies will be my catalyst; so I add these antibodies to this A. So, now, the transition state from A to B looks very similar to the intermediate according to Hammond's postulate; so the antibodies will now stabilize the transition state. If the transition state is stabilized, that means, the activation energy will go down which implies that the transition state will be easily converted into the product B. So, that is the whole idea of this topic of synthetic biology. Synthetic biology has other domains. We are just concentrating on how to make artificial enzymes or enzyme like systems; it is through our immunological response.

The whole basis is that you have to use the immuno-response that is available in a living system like mice or human because our immune system protects us from the infection or invading agents from the outside. Now there are certain problems that we will have to discuss. We will also discuss that what are antibodies and how they look like.

And then we will also talk about the issue that does any foreign molecule generate antibodies in our system or not? So, these are very important because some antigens or some molecules; if injected into the body, may or may not generate antibodies. The classic example is that when we become sick, what we do? We take a medicine. What are medicines? These are small molecules; you will never find a medicine whose molecular weight is very large; that is not possible.

What you do, you take only small molecules as medicines; and if I take the small molecules. If there is immunity developed against that small drug molecule, then the drug will not work drug. So; that means, antibodies are not generated against small molecules. So, that is a stumbling block here; although the theory looks very good that I should take the inhibitor, inject it into the mice, isolate the antibodies that should catalyze the reaction,; but antibodies are not generated against small molecules.

When you talk about these reactions, these reactions are basically between small molecules, not involving any large molecules. So, that we have to discuss; that means, small molecules are not immunogenic. What is immunogenicity? If some molecules fail to develop immune response, then that is not immunogenic. And if the invading molecule elicits an immune response inside the body, and consequently generates antibodies, then those molecules are called antigens. So, what the small molecules does in the body? The small molecules sometimes give response which are called allergic response. Many of the small molecules when they go into the body, it starts itching or some skin rashes show up; these are allergic reactions. Like some people are allergic to penicillins, but nobody will develop an immune response against these small molecules; so, small molecules are not immunogenic.

So, forget about isolating antibodies by injecting small molecules. So, you have to follow some other strategy.

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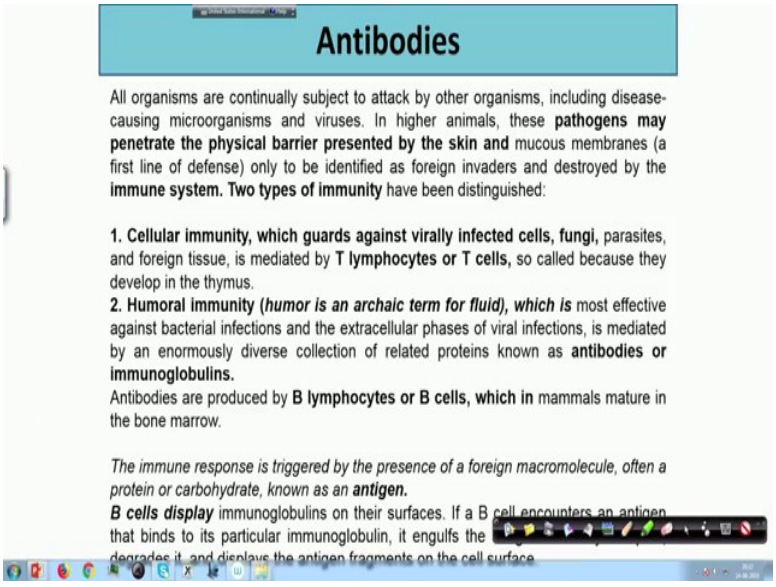

I think we have already told you that antibodies are basically generated against antigen invasions. It could be organisms, it could be virus, it could be large molecules; antibodies are generated and this process is called the immune response. Now, there are two types of immunity: one is called cellular immunity and the other is called humoral immunity. Cellular immunity guards against the virally infected cells or fungi parasites and foreign tissues. So some immunity comes through cellular processes.

Our cells presents inside the body, specially what is called the T cells or specifically the T lymphocyte cells, are associated with cellular immunity. T stands for thymus because they are generated in the thymus. These T cells through a cellular immunology processes guard against the virally infected cells; that means, the cells or tissue where some viruses, some fungi or parasites have entered. The other type is the humoral immunity. The body fluid is called humor.

Humoral immunity is basically most effective against bacterial infections and cellular phases of viral infections. Thus basically, there are two types of immunity; one is cellular immunity and the other is humoral immunity. When there is humoral immunity, that actually is mediated by antibodies or they are also called immunoglobulins.

So, we are interested in the second type of immunity: the humoral immunity. Antibodies are produced; so, who produces the antibodies? It produced by B cells or typically called B lymphocytes; B cells are actually present in the bone marrow. These B cells are the ones which generates the antibodies. It is the cell which makes the proteins, the enzymes. I already told that what is immune response? It is triggered by foreign molecules and this is called antigen.

Foreign molecule is often a protein; it could be a big protein or it could be a carbohydrate; that means, large macromolecules can only act as an antigen; small molecules cannot. Small molecules can invade our immunological machinery because they are very small; they are not noticed.

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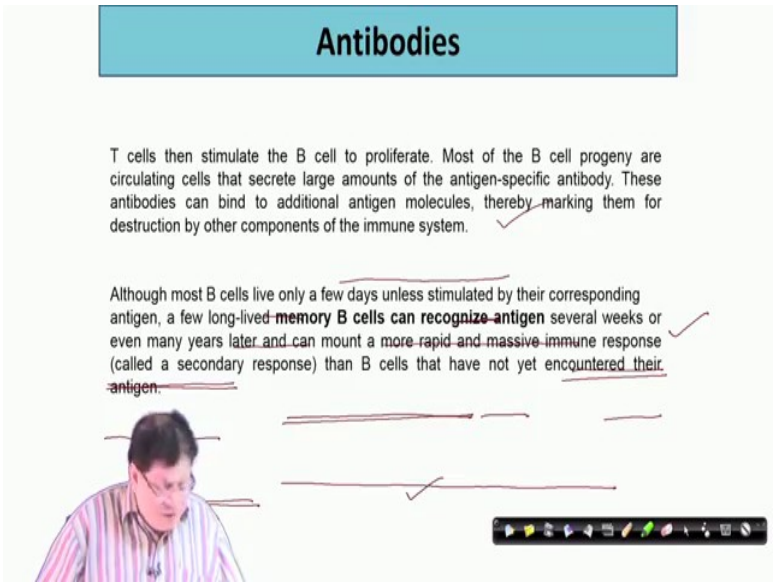

So, the big question is how to generate antibodies against the small molecule? So, that is a big challenge. There is another problem if you have some big molecule entering into the body.

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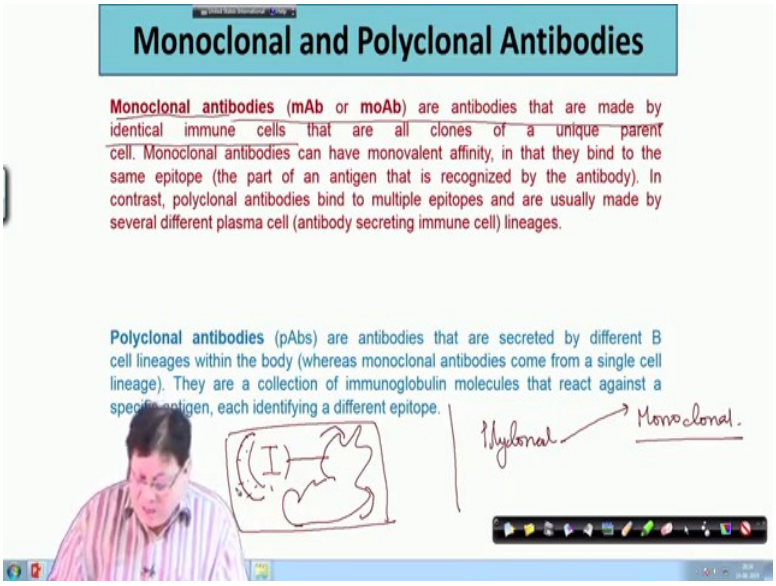


Suppose, this is the shape of the big molecule entering into the body; so now, some process signal will go to the B cells that something has come and this is the shape and structure of the big molecule.

Then this B cells starts producing this immunoglobulins or antibodies. All these antibodies are against the specific antigen. Antigen is a big molecule. There are different types of antibodies for a given antigen. So, these antibodies are going at different sites of the antigen and binding.

Thus the antibodies are attacking the same molecule, but targeting different sites. So you have a collection of different types of antibodies. Suppose I have different balls; 5 red balls, 4 blue balls and say 7 green balls; all these balls are like footballs. Suppose, you can play football with any one of these balls. We say that we have a collection of polyclonal balls; this means you have a collection of different types of balls, but everything is targeted towards playing football.

Similarly, here these antibodies are also targeting the same antigen molecule, but at different sites. So, if you take the blood and try to isolate the antibodies against a particular antigen; you will see that it has got a variety of antibodies. Their common point is that all of them are attacking the same antigen, but at different sites and also their efficiency of binding will also be different.

So, these are called polyclonal antibodies; polyclonal antibodies are antibodies that are secreted by different B cell lineages within the body. So, we have different types of B cells; one B cell will generate antibodies which go to a particular site, some B cells will make another set of antibodies which goes to other sites of the antigen. So, different types of B cells, will together make a set of polyclonal antibodies.

So, now, what you do? Suppose we want to make antibodies against the intermediate, but we know that the intermediate does not generate any antibodies because it is a small molecule. So, to generate antibodies, what you do? You attach the intermediate structure to a large molecule.

So, you conjugate the intermediate to a large molecule. If you do that, antibodies will be generated which recognizes the antigen (the whole ensemble comprised of the intermediate and the large molecule conjugate) at different sites. Suppose that some antibodies may be there which recognizes this site, some antibodies will be there which recognizes some other site.

But suppose there are some antibodies which recognize the intermediate which is a part of the whole system. Then these are the antibodies that will now stabilize the intermediate; that means, in they will stabilize the transition state. So, the theory is again not very complicated; if you want to generate the antibodies against small molecule; so what you do?

The small molecule has to be attached to a big partner (a macromolecule) like a protein. And then this whole thing is injected and you get a set of polyclonal antibodies and if you are lucky, then some antibodies are there which recognize the intermediate structure of this whole ensemble.

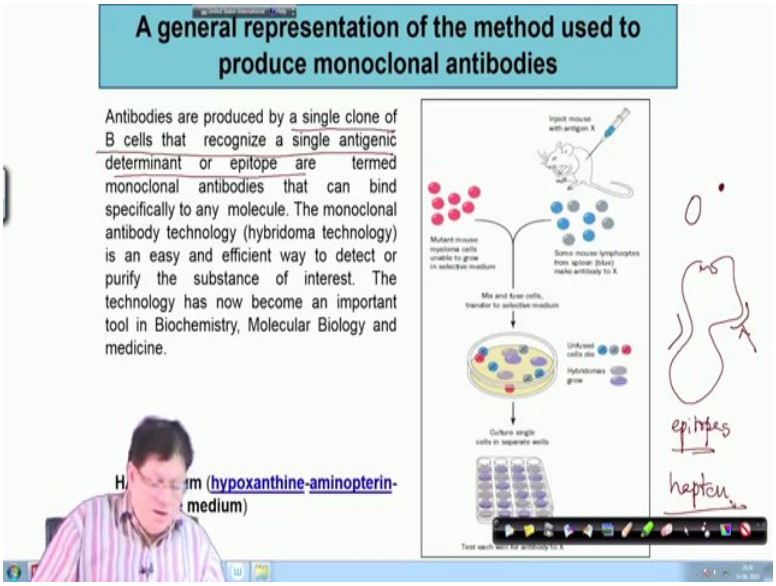
Then if you can isolate those antibodies, they are going to catalyze your reaction because only they can recognize and bind to the intermediate part and thus they will be the catalyst. Some definitions you should also know. What are monoclonal antibodies? I have already told. They are the antibodies that are made by the same type of B cells making only one type of antibody.

That means, if I have those red, blue and green balls; I separate only the red balls from there; then this red balls are all identical; so that is a monoclonal set. Similarly, I have different sets of antibodies; some goes and binds here, some goes binds there and some bind to the intermediate.

From this polyclonal antibody set, I need to have some mechanism to separate it into monoclonal antibody which recognizes only one site of this whole thing. And these monoclonal antibodies come from only a particular type of B cells, other type of B cells will generate other type of antibodies So, now just to summarize that; in order to create an artificial enzyme; what is to be done?

You draw the mechanism of the reaction, draw the intermediate and if you have a similar structure like intermediate or if the intermediate is quite stable; then you attach it to a large molecule which is called the carrier molecule. Because that is carrying the intermediate along with it and then you develop polyclonal antibodies against this whole system and then your task is to separate this polyclonal into monoclonal antibodies and so that this monoclonal set recognizes just the intermediate .

So, this separation and this attachment (bio-conjugation) is required. (Refer Slide Time: 37:22)



I already told that a single clone of B cells will produce only one type of antibody; only one site of the antigen will be recognized. Why antibodies are not generated against small molecules whereas they are generated against large molecules? Because large molecules have characteristic surface where they have different geometries at the surface which can be noticeable. But if you have small molecules, as compared to a biological system, they are considered as tiny dots. So, there is no characteristic feature of the surface of the large molecule.

If that is not present, then it will be difficult for the antibodies to bind. So, you should have some characteristic feature on the surface. Like this is a bent one, so you can have a complementary structure for that; you can have a complementary structure for this site also. That is why large molecules are recognized and not the small molecules because small molecules are basically tiny dots; they do not have any structural pattern.

Now, these sites of the antigen where the antibodies bind are called epitopes. Epitopes are the specific binding sites because not all portions will bind to the antibodies. They are specific sites in the antigen where antibody binds. These small molecules which is attached to the large carrier molecule are called is called haptens. .You are developing antibodies against these small molecule.

Haptens are basically small molecules which mimic the intermediate and when it attached to the carrier molecule; it triggers the formation of antibodies which recognizes the small molecule.

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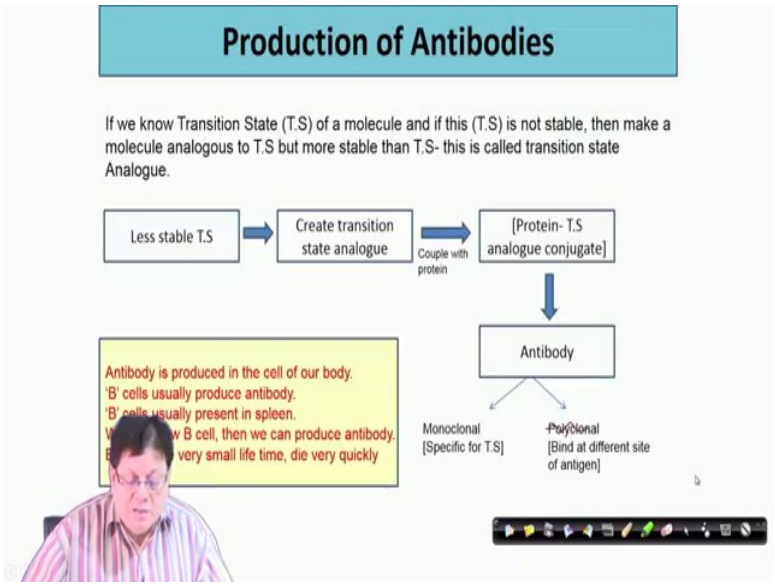

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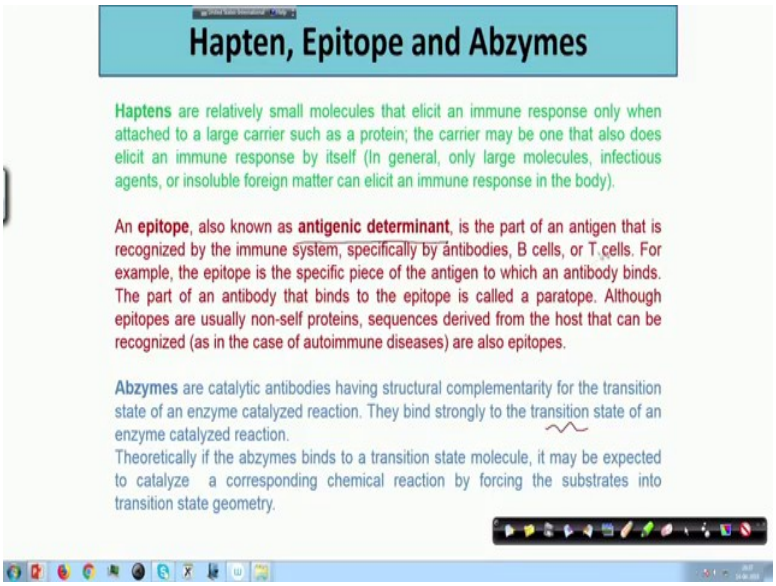

Haptens are small molecules that elicit immune response only when attached to a large carrier such as a protein. The carrier may be the one that itself can create immune response and it can create immune response even when attached to the small molecule.

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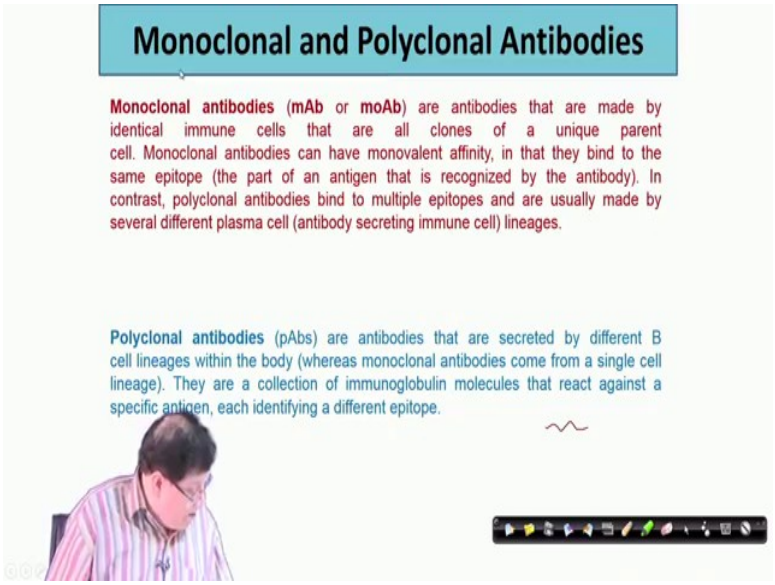

There is another name for epitope; that is called antigenic determinant. It is the part of the antigen that is recognized by the immune systems specifically by the antibodies the B cells or the T cells.

And this epitope is the specific part, actually it is that piece of the antigen, to which antibody binds. Now the things are little bit easier as we proceeded. So, basically what you have? You have to have an intermediate attached to a carrier molecule and then isolate the monoclonal antibody from the polyclonal system. And this monoclonal antibody should recognize the intermediate and have a binding affinity towards it; then that antibody will become a catalyst.

Because catalyst stabilizes the transition state and transition state mimics the intermediate. Linus Pauling won two noble prizes; first one was basically for the structure of proteins; the α helical structure of proteins; he did the structure elucidation of insulin. So, at that time he studied many enzyme reactions; enzymes mediated catalysis of various biochemical reactions. He wrote a very famous book called Nature of Chemical Bond.

This volume was the same volume which gave the hybridization theory. So, at that time, he said that enzymes stabilize the transition state and one day may come that people can develop enzyme like systems which recognize the transition state and then it will catalyze reactions. So, that will open up new avenues for organic synthesis.

Thank you.