Organic Chemistry In Biology And Drug Development
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Lecture - 20
Synthetic Biology (Contd.)

Welcome back to this course on Organic Chemistry In Biology and Drug design. Last time if you remember, we were discussing some of the aspects of synthetic biology; We were specially interested to know that whether enzyme like molecules can be made to catalyze a particular reaction

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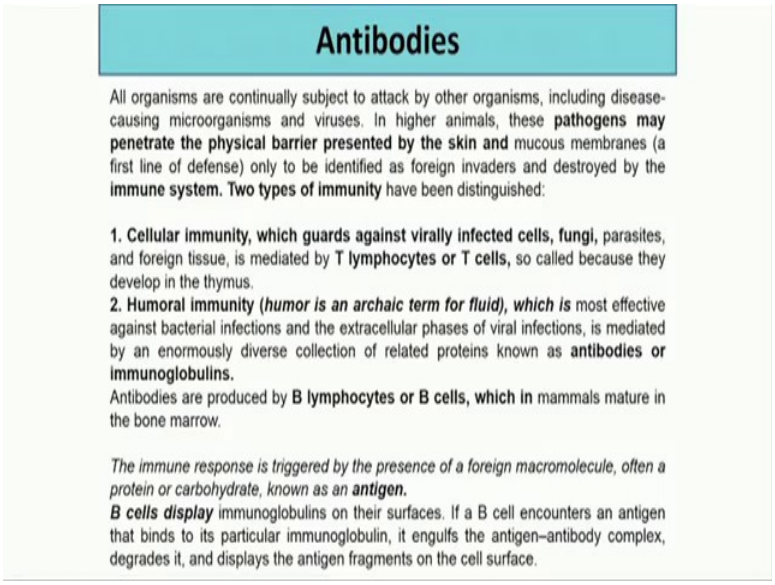

I told you the limitations of the enzyme chemistry, the limitation is basically the number of enzymes which nature has provided us. Because if there is an organic reaction where there is no counter part of an enzyme, then you do not have any enzyme catalysis for that reaction.

So, people have thought about knowing the principles of enzyme catalyzed reactions, Then we have to consider that whether that principle can be adapted and whether the biological system can be manipulated to generate enzyme like molecules that can catalyze, virtually any reaction; provided we know the mechanism of the reaction and the geometry of the transition state for that reaction.

We know that enzymes catalyze reactions by stabilizing the transition state more than the substrate or the product; that means, the transition state geometry is complementary to the enzyme active site. This complementarity means electronic complimentary as well as steric complementarity. In biology, there is one process which guards us against infections and it is constantly acting as a surveillance mechanism to protect us from environmental systems, which are harmful to the body. This is called immunity. We have immunity as long as we are we are living.

So, we have immunity that is guarding us against infections. Now immunity is a response that is created in the body against some invading organism or invading molecule, that is not known (foreign) to the system; that means, the invading molecule is not self-molecule. So, it should be a foreign particle and this foreign particle is known as antigen.

And what happens in case of humoral immunity? Humoral immunity is basically mediated through the proteins that are flowing in the blood cell. On the other hand, cellular immunity is imparted by the T cells and it guards us against the infection causing foreign agents. . In humoral immunity, antibodies are made depending on the structure of the antigen. And this antigen has the exact complementarity to the antibody or vice versa.

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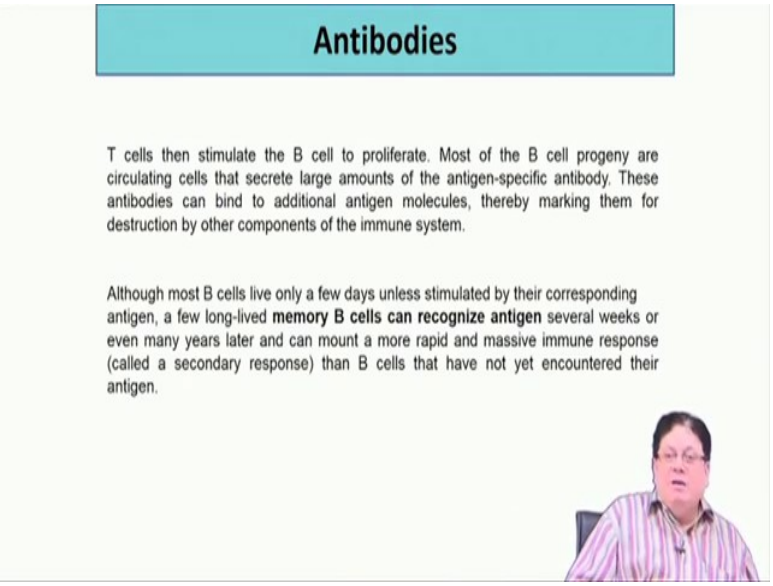

People thought about exploring the concept of matching of antigen antibody to make enzyme like molecules.

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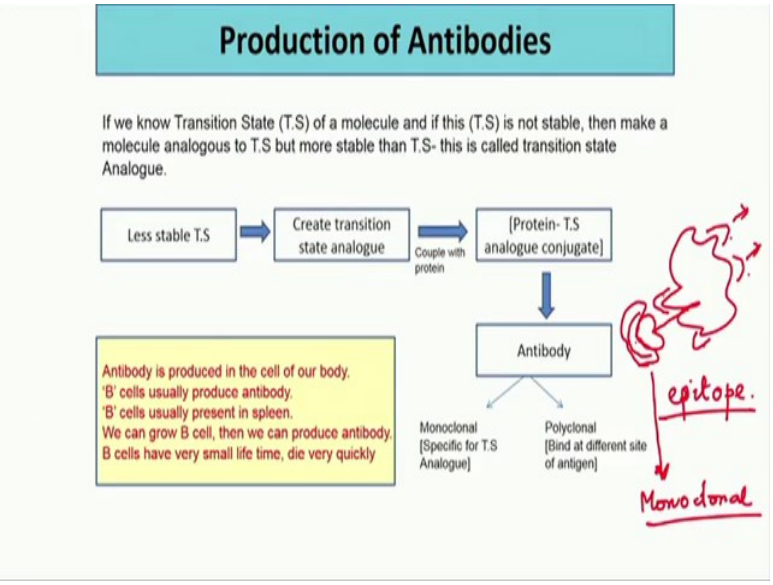

And the theory behind this is shown here. See if you know the transition state of a molecule and if this transition state is not stable at all (which is always the case), then we can make a molecule which is analogous to the transition state because transition states are transitory in nature, they cannot be isolated. So, you make an analogous molecule whose geometry and electronic character is very similar to the transition state for the reaction and this analogue is more stable than the transition state and this is isolable. This is called a transition state analogue.

So, transition state analogues are basically molecules which are electronically and sterically very similar to the transition state of a particular reaction. So, you produce antibodies which recognize this transition state analogue. You basically write what is the transition state analogue possible, synthesize that molecule.

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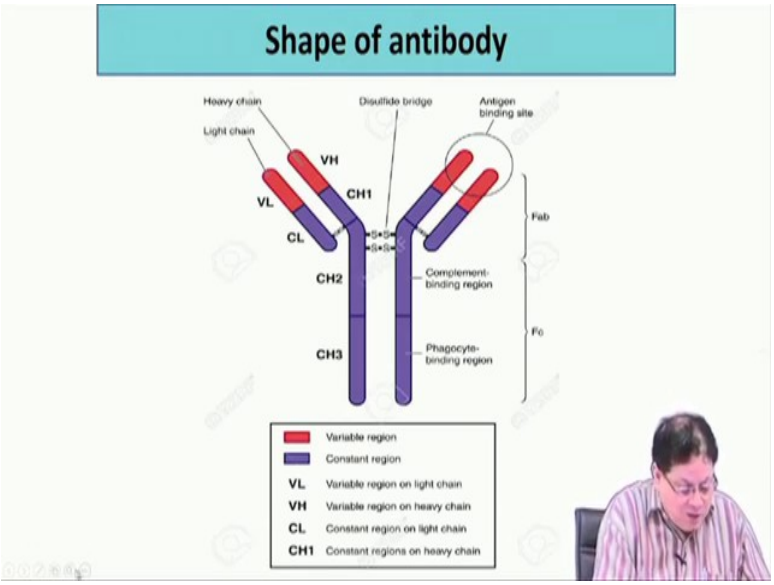

Last time I said that small molecules do not elicit immune response because small molecules are not captured by our immune system. So, there is no immune response because they are very small.

So, in order to have immune response for the small molecule; you attach the small molecule through a linker to a large molecule (say another protein). So, you have a transition state analogue protein conjugate and then you inject that into an animal like mice, and then the mice will develop antibody because they also have the immune system like us; the mice will generate antibody and these antibodies are called polyclonal antibodies because they recognize different parts of the antigen which is comprised of the conjugate between the carrier and the transition state analogue. So, now, when the antibodies are generated, some antibodies will bind here, some antibodies will bind there, some antibodies may bind there. So, it just looks for the characteristic shape of the surface and accordingly it generates the antibody.

It can generate the antibody for the transition state analogue also. So, you have a collection of different antibodies and these antibodies have one thing in common; they go for the same antigen; but they bind at different sites and their binding affinities are also different. These are called polyclonal antibodies.

So, if you have polyclonal antibodies, some of the antibodies in this polyclone may be recognizing your transition state. Now remember this site where the antibody binds, we have a name for that; it is called epitope. Epitope is the site where the antibody binds. If the epitope happens to be present in the transition state analogue, then you will get an antibody generated against it.

Now the next target or the task is to separate this polyclonal set where few of the antibodies may be recognizing the transition state and you are only interested in those ones, because those are the ones which will catalyze your reaction because they are recognizing your transition state analogue; that means they are going to catalyze your reaction because the transition state energy will be lowered by stabilization with the antigen antibody interactions.

So, out of this collection of polyclonal antibodies, you have to separate the individual antibodies. You are not interested in antibodies which bind to the protein molecule; you are only interested in the antibody that binds to the transition state analogue because that is going to be your catalyst.

These will be monoclonal antibodies because that goes only to one site; they are binding in the same site with same efficiency. So, these are called monoclonal antibodies. So, I think I have a definition here polyclonal and monoclonal antibody.

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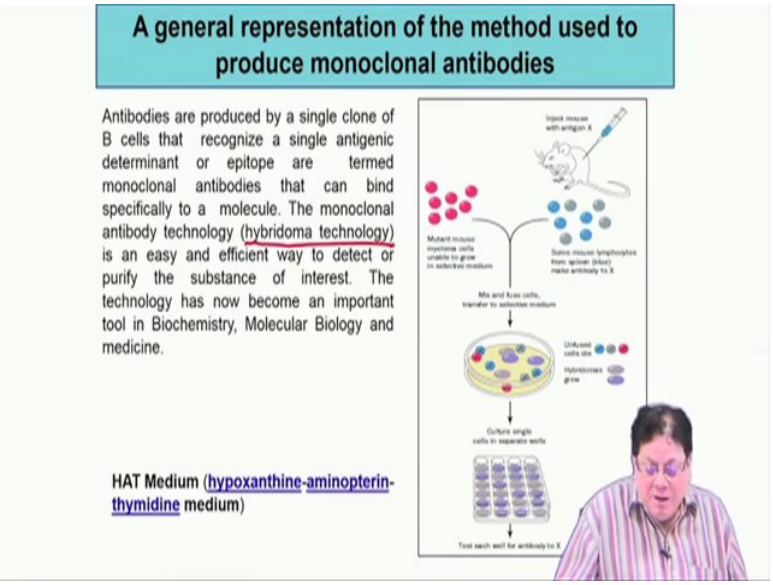


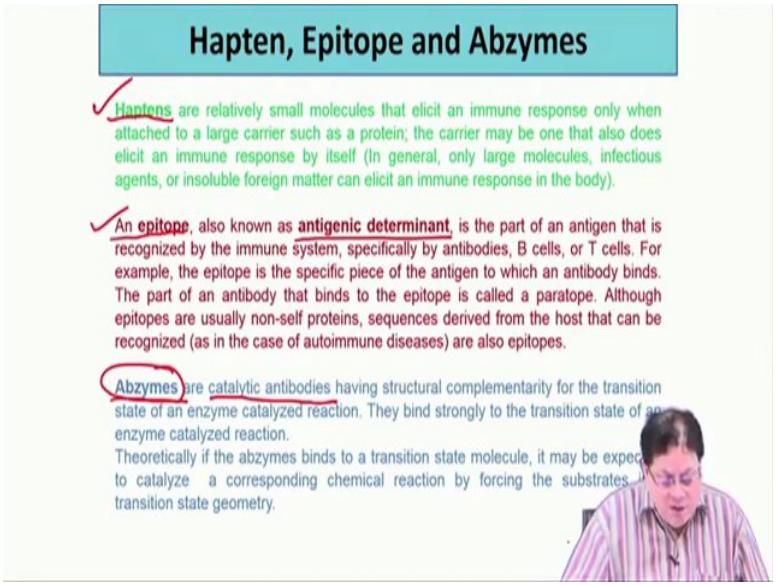
One type of antibodies is produced by a single clone of B cells. Clone means virtually nothing, but photocopies of something. Like you have a photocopy of your mark sheet or certificate. So, the collection of photocopies of the same mark sheet are called clones. Now if you have a mark sheet for the higher secondary exam and this is the mark sheet for your 10 exam; and if you have mixed up all these mark sheets, then you have a polyclone. The more number of exam mark sheets that are mixed together, the more polyclonal the system is.

So, from that system of polyclones, suppose you have to now separate the photocopies of the original mark sheet of your class 12. So, that is the basic idea behind the monoclonal set. Monoclonal set means, they are all same type. So, they are produced by the same type of B cells because there are different types of B cells. One type of B cell will produce one set of antibodies, another type of B cell will produce another set of antibodies and then there are different kinds of B cells.

So, they will all produce different sets of antibodies and all these different sets of antibodies ultimately target the invading organism or the invading chemical species. Since they are polyclonal, so, now, you have to separate it into monoclone. That is the challenge. Now there is a technology which is called hybridoma technology. The hybridoma technology allows us to separate and isolate the monoclonal antibodies from a polyclonal set.

And this is a very important development in molecular biology. This is a very important tool in biochemistry, molecular biology and also in medicine. If you want targeted drug delivery, then you attach a monoclonal antibody which targets something (some particular cell) which you are interested to destroy. It goes and binds to that cell and then the drug is released leading to the cell death. That is one aspect.

Another aspect is its use as a diagnostic tool. If you want to really know the presence of certain things in the blood, in minute amount, you can use monoclonal antibodies. There are techniques where there is recognition followed by the change in color. (Refer Slide Time: 13:42)



Let us just have a quick review of these definitions. Hapten is the small molecule; that means, basically the transition state analogue, which itself cannot elicit immune response but when attached to a large molecule like a protein, it can generate antibodies which recognize the small molecule. The transition state analogue will be called a hapten.

An epitope is the site where the antibodies bind; or this is also known as antigenic determinant. The third term is abzymes; abzymes are these enzymes which are also called catalytic antibodies; that name has come from antibody as enzymes. So, that is why the name is abzyme but it was also known initially as catalytic antibodies; but to abbreviate it, you have now the name given as abzymes. Thus they are antibodies functioning as enzymes.

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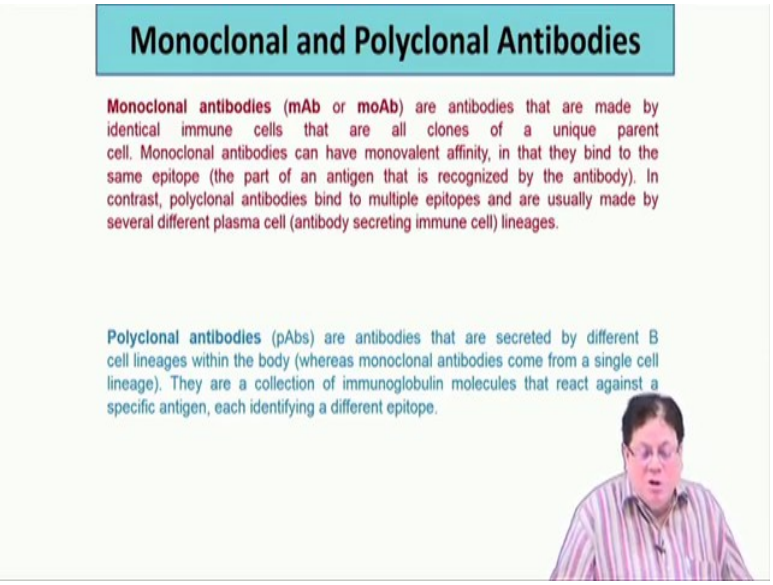

We have discussed these monoclonal and polyclonal antibodies, next is that how do you really isolate the monoclonal antibodies? Before we go into that discussion, let us discuss the structure of the antibodies. The structure of the antibodies is shown here. Antibodies are basically tetrameric protein molecules. Since they are proteins, so they are made up of amino acids. It has a Y shaped structure. There is one bigger chain that is called the heavy chain and there is a smaller chain called the light chain.

These two chains are attached by disulphide bonds and this red part is the antigen binding site. So, the antigen actually binds here and this is the variable part, because different antigens have different characteristics. Accordingly the variable part of the antibody will also change. So, the most variable part is this antigen binding site which is at the top. So, this is just a cartoon picture representing the shape of an antibody.

Now, let us come to the topic that how do we isolate the monoclonal antibodies.

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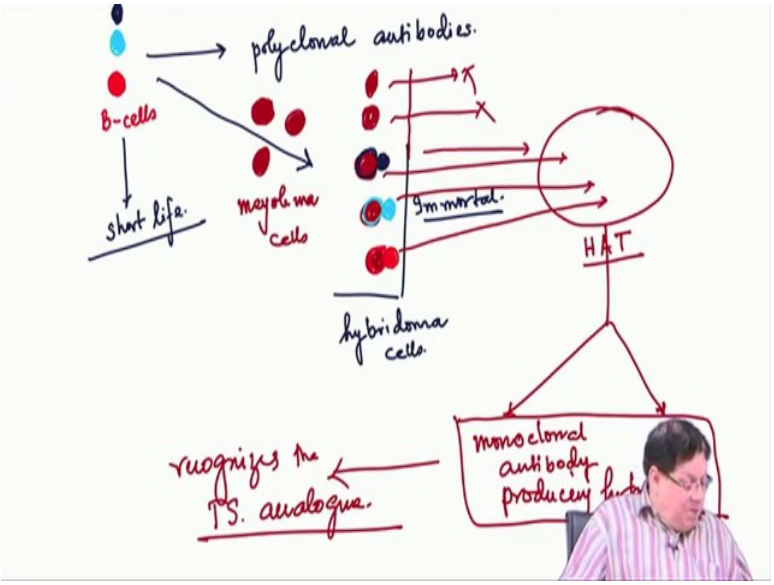

So, we have a challenge now; that is to separate the monoclonal antibodies from the polyclonal antibodies Polyclonal antibodies are made by the B cells. When you inject the transition state analogue coupled with the protein into the mice, antibodies are generated. You do not isolate the antibodies because they will be very small in number; you isolate is the antibody producing cells (B cells).

You have different types of B cells because they are making polyclonal antibodies. Some B cells produce one type of antibodies and there will be some B cells which will make another type of antibodies.

So, there are different types of B cells. Unfortunately these B cells are very short living as they have very short lives. So, if you isolate them, they are going to die; you cannot really multiply them and unless you multiply them, you will not get a supply of these antibodies.

Remember each of these B cells are producing antibodies, but if you want to get a supply of antibodies, you have to culture them; you have to grow them; but unfortunately they have a short life and they die very quickly. So, what do you have to do? You have to make them somewhat immortal, so that they do not die.

To do that, you have to take the help of something which is extremely dreadful (if it is present in the body); but we will see the application of those type of cells. When there is the presence of cancer cells, which are also like myeloma cells, they cannot be destroyed very

easily; they always replicate; they are virtually immortal. Unless you bombard them with UV light or some chemotherapy or radiotherapy, cancer cells are virtually immortal; they will grow continuously; that is the problem.

So, that is called the malignancy where the cells grow uncontrollably. They grow continuously and hence they are virtually immortal. So, now, what you do? You take all these B cells and the collection of these cancer cells which are also called the myeloma cells.

So, when you take all these together and put a lot of pressure in a solvent (say polyethylene glycol), what happens? Like if you take two blocks of ice and then press them and release, the two blocks of ice become one, this is the process called regelation and that you have read in high school physics.

So, here very similar thing happens. If you take all these cells and then force them together, what will happen? They will fuse with one another. I think I have to take different colors; see some are fused with these red ones. So, you have a cell now which will look like this; this is the cancer cell part of it and the other part of it is your B cell.

So, now your cell will look like this. Again it is another B cell which is fused to the cancer cell and I will just write the third one also. So, that will also fuse with this. So, you have now these mixed cells which are basically the B cells fused with the myeloma cells (cancer cells); and these cells are called hybridoma cells because they are the hybrid cells. Interestingly because these hybridoma cells have the cancer cells in it so, they are basically immortal now. But remember, when I do this fusion, some of the cells may not fuse at all because there is no guarantee that all cells will fuse.

So, some will remain as just the myeloma cells; nothing else; and if there are B cells, then those B cells which are not fused will die automatically. So, you do not have to do anything. Now, you culture this in a plate and in a special medium which is called the HAT (Hypoxanthine-Aminopterin-Thymidine) medium. It is a special medium; this medium selectively does not allow only the cancer cells to grow. So, it must be having some antitumor agent here. But this medium allows the other cells to grow; the hybridoma cells will grow here.

We are not going into much of the details. You have a polyclonal set here, since some of the cancer cells are fused with this one, some will be the blue, some will be the red in color.

Now there is a process by which you can separate this monoclonal antibody through producing hybridoma cells. I can talk about the principle behind this. The principle is that, once these cells grow, you take these cell collection of polyclonal antibodies and then you do dilution; that means, suppose you have 100 cells in a test tube which are polyclonal; but if you half-dilute, you have 50 cells and then upon further half-dilution, you have 25 cells.

So, at some point of time you will get a cell which is only particular type of B cells. Once you have that, they are the monoclonal antibody producing hybridoma cells. Basically it is a high dilution technique and ultimately you can separate these hybridoma cells into monoclonal sets.

Remember, each hybridoma cell is immortal because they are fused with the cancer cells. So, they have the property of the cancer cells at the same time, they will produce the antibody which will bind to the antigen that you have targeted. So, this is very interesting that how you separate this monoclonal antibody producing B cells. Thus ultimately you get a monoclonal antibody which recognizes or which binds to the transition state analogue through proper recognition. You take hybridoma cells, the advantage is that you can always grow the cells whenever you want. You grow these cells and you can get grams of these monoclonal antibodies; So, you keep it in the fridge; whenever you require, you take a specimen of this hybridoma cells, do fermentation, and then isolate because they will generate the antibodies and you can isolate the antibodies.

And these antibodies are the ones which will catalyze the reaction that you are talking about. I hope the principle is clear because upto this part is biology; then we will go to some examples of actual chemical reactions which are catalyzed by this technology using abzymes.

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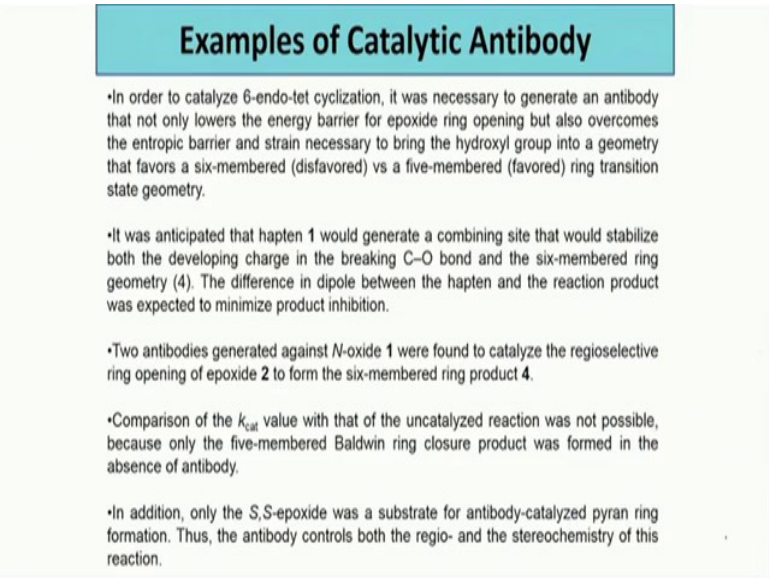

In the beginning, I told you something that this catalytic antibody discovery is extremely important in the sense that virtually any reaction can be catalyzed but there is one requirement that the mechanism for that reaction has to be known. There is another interesting part of it. Those who have studied organic chemistry, know that many of the reactions are called regiospecific reactions, stereoselective reactions, or chemoselective reactions.

There are different kinds of reactions which have particular selectivities. There are different types of selectivities: regioselectivity, stereoselectivity or chemoselectivity. Now some reactions are actually against the rules of selectivity. So, those reactions are called disfavored reactions. Suppose you try to do a reaction where there is a possibility of a six-membered ring formation or a five-membered ring formation. Usually we think that 6 membered ring is more stable.

So, six-membered ring should be formed; but the six-membered may be disfavored; disfavored means the orbitals are not aligned to do a bond forming reaction to lead to a six-membered ring.

So, there are certain reactions which are called disfavored reactions and this catalytic antibody technology can be used to even catalyze disfavored reactions which were otherwise not possible. So, these are the various advantages of this strategy.

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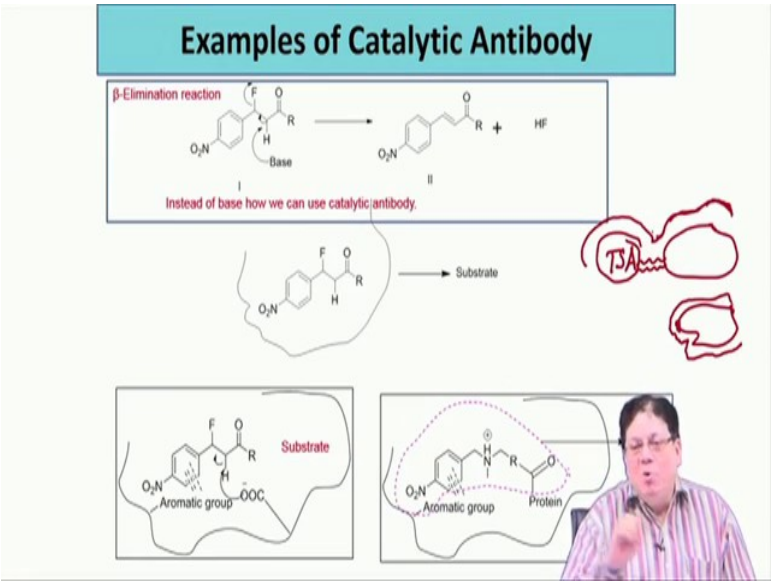

And again I say that if you have isolated the monoclonal hybridoma B cells producing the catalytic antibody, then you are through; because that is immortal and you can make grams of the stuff whenever you want. It is worthy to remember that these are not enzymes, they are catalytic antibodies acting as enzymes, better known as abzymes. These abzymes actually have much better bench stability or shelf life than other enzymes.

Their shelf life is quite high. So, you do not have to take extreme precaution to preserve the catalytic activity of these abzymes. Now the question is that how do you generate the catalytic antibodies or how do you design the catalytic antibodies to catalyze certain reactions? I have told you that you have to generally make the transition state analogue which are stable, attach it to a protein and this protein is actually called the carrier molecule; but remember that there is also a linker that needs to be used.

This is the transition state analogue; usually you should use a linker because if the transition state analogue is embedded right on the surface, then it may not be noticed by the surveillance system (which is your immune response). See our immune system is trying to monitor what has come. Whenever some foreign particle comes, our system just hovers around the surface and then sees what are the different geometries, what are the different amino acid residues on the surface and accordingly the information is passed on to the B cells and the B cells make the antibodies.

So, what happens if your protein and transition state analogue is embedded here? The surveillance system may miss the transition state analogue because it is right on the surface. So, it may not find this interesting and interpret that this has no characteristic surface here; but if it is extended by a linker, then this will definitely find something which it can sense or recognize.

So, now, it may be a characteristic epitope. So, basically you have to design a hapten and that should have an epitope. If the hapten does not have any epitope, then no antibody will be generated against the hapten.