Immunology
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Lecture – 11
Structure of Antibody

Hello everybody, so hope you are enjoying the course. So, today we are going to discuss about the structure of antibody. So, in structure of antibody we already know what is antibody in previous classes. We are going to discuss the structure of a typical antibody molecule why I am talking typical antibody molecule you will understand later on.

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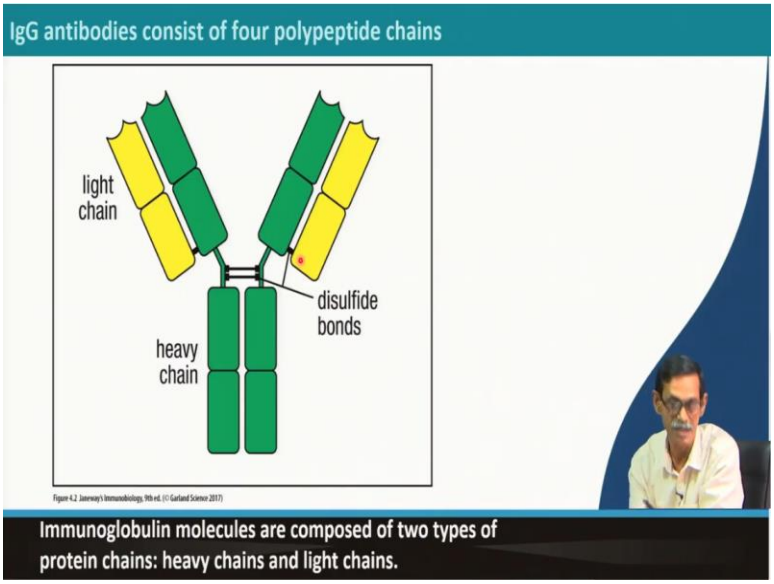

Seen antibody there are 2 types of polypeptide chain one, the big one which is green here, which is the green one is the bigger one and another is yellow one the small one, So the big chain we call heavy chains and the small chain we call light chain depending on the molecular weight. So, if you see this picture, I mean it is the cartoon of the antibody molecule, you can see the 2 heavy chains are connected with 2 disulfide bonds and one heavy chain and light chain is connected with disulfide bonds.

So, immunoglobulin molecules are composed of 2 types of protein chain one is heavy another is light, heavy chains and light chains. So, 2 heavy chain 2 light chains composed antibody molecule which looks like Y, English letter Y.

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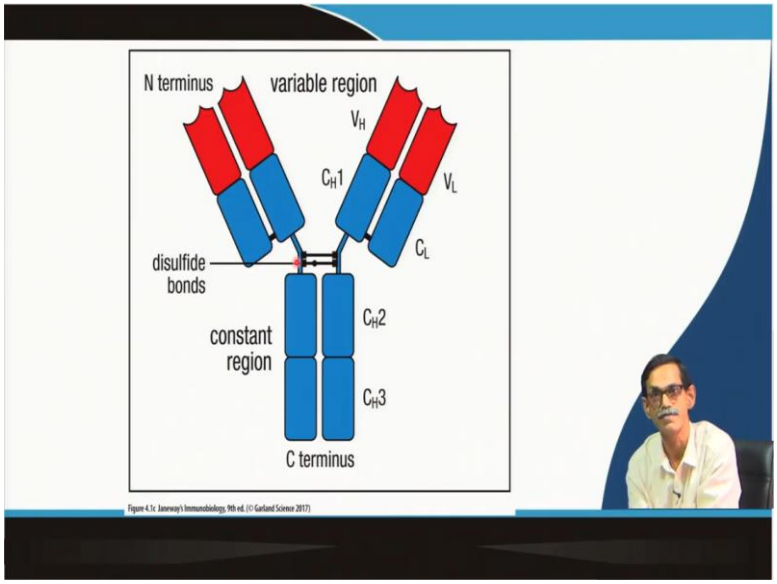

The same picture a little different I can understand from the colour, not only the colour, you see there are domains are named here. You see both light chain and heavy chain, the upper part is red and rest of the part is blue. Why this red? Red here you see it is written V L and V H is red is indicated the variable region, because in antibody this region is responsible for antigen binding. So, this region is responsible for antibody antigen binding and as there are so many varieties of antigens, we should have a different antibody which can interact with different antigens.

So, this region is responsible for antigen binding, so this part must be maximum variable. So, this and it is true if you find 2 different antibodies you will find the blue region is almost similar, but the red region is different. So, that is why these domain and if this is a polypeptide chain, this is the N terminal and this is C terminal in case of light chain, this is N Terminal, this is C terminal. So, the N terminal region of heavy chain and N terminal region of light chain basically determines the antigen binding specificity.

And this is the most variable regions for both the cases it is called variable region is written here. And it is designated by V H that means, heavy chain variable region and V L stands for light chain variable region and same way the blue region is known as constant region. Blue region is known as constant region and constant region also if you see there are 3 domain in case of a

heavy chain and one constant region in case of light chain. So, constant region of light chain is designated as C L and heavy chain constant, region or domain is designated at C H.

Now there are 3 domain it is called C H 1, C H 2 and C H 3, C stands for constant, L for light, C constant H for heavy and 1 2 3 is 3 different domain. So V L, C L, V H, C H 1, C H 2, C H 3 and this one such unit are joined together and 2 such units are joined together by the disulfide bond.

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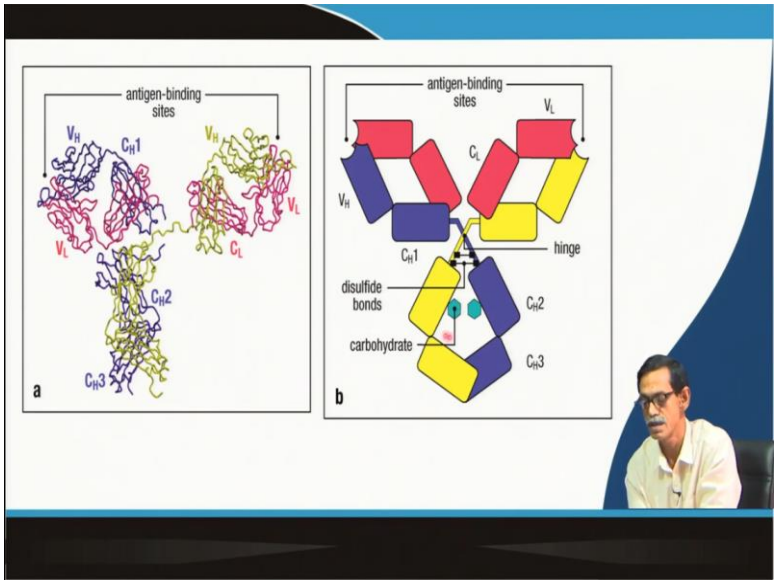

But it is not as straightforward that what we see last time if you see carefully, this is actually the structure. So, it is not just like to stand is just linked here in this region like that. It is basically they are cross like this so, they are cross like that and 2 light chains are going to join here. So, if you see the 3 dimensional figure it is just not planner. It is kind of cross crossing each other. And here, you see these 2 heavy chains, the violet and the yellow; they cross each other and light chains are in 2 different ways.

So, if you see this thing it will do like this. So, it is a crossing here and it can have 2 binding site at 2 positions. And this is and this structure, whatever the cartoon drawn here, it is derived from the crystal structure discovered; I mean the crystal structure after the discovery or identification of the crystal structure. So, this is a cartoon of the crystal structure and here the hinge C H 1, C H 2 everything is mentioned.

What you can correlate with the previous figures and these hexagonal things are carbohydrates, all the antibodies are highly or heavily glycosylated. There are I mean different antibody have a different glycosylation pattern and this glycosylation is also important for the effector function, because some antibody is more favorable for complement action, some antibodies are more favorable for optimization. So and this sugar is very important and but for this particular cartoon, we should remember that the constant region or sometimes in the variable region also that it is glycosylated. So, this is the cartoon of antibody structure based on the crystal structure.

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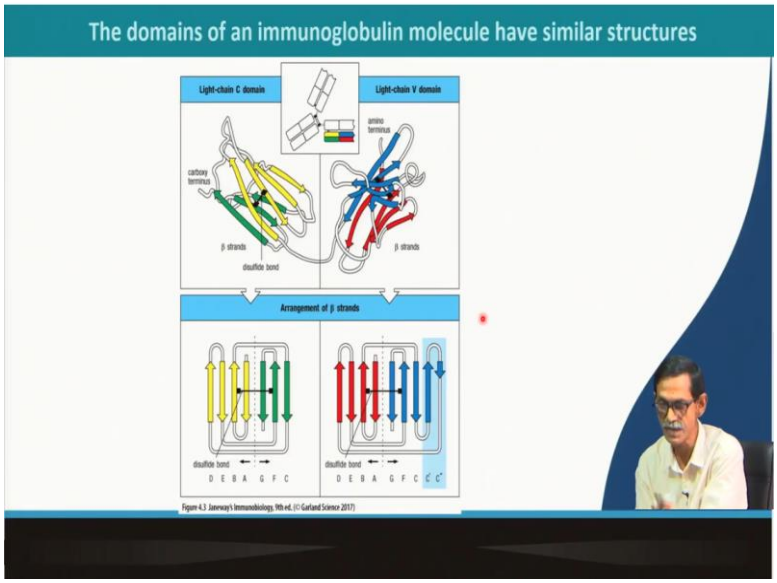

But now, if I see our if we want to see how these domains are organized, according to protein level structure. So, the same domain if you see, this is the antibody and this antibody 2 domain. This is the variable domain and this is the constant domain of the light chain and in this case these 2 if you see more detail what we will see is that these particular, say the variable region of the light chain is composed of mostly the anti-parallel beta sheet.

Anti-parallel beta strands or beta sheets are formation and there are lots of loops. So, variable region of the light chain if you see there are 2 parts in this cartoon, blue and red here is blue and red is mostly the beta strands red indicating the red zone of here and blue indicating the blue zone of here. Similarly, if you see the constant domain of light chain, the C L is also very similar.

It also has mostly beta strands and green and yellow is indicating the green and yellow part of this cartoon our beta strand and rest of the loop, and if you make them straight arrangement of the beta strand how it looks like you see they are anti-parallel one goes this way another this way that these, these, these and these pattern, you see these 2 domain they are very much looks alike it is the anti-parallel beta sheets and not only that if you see the heavy chain, they are variable region their C H 1, C H 2, C H 3 are very very similar.

So, the domains of immunoglobulin molecule are similar structure which is mostly constitute of anti-parallel beta sheets and some loops. So, what are the loops? I will come later.

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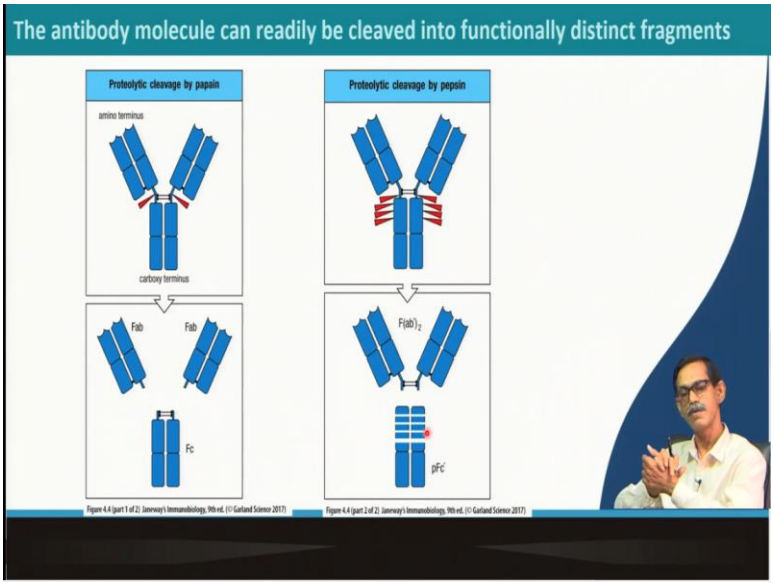

So, before that just for now, that all the domains are having similar structure. So, summarizing each domain or similar structure, there are one variable domain in light chain and one variable domain in heavy chain one constant domain in light chain and 3 constant domain in heavy chain and before the molecular biology or the recombinant technology discovered or the all the techniques now we know we can do many things now.

We can know the sequence we can manipulate the sequence, but before moving all this technology or all this information progression of all this information, what was known that the structure of antibody. I am not going to go detail how it is discovered, but very interesting thing I

mean just interesting in general, so, you that all antibodies, all varieties of antibodies can be cleaved in a functionally distinct fragments.

Antibody molecule, in general, some peptide previous IDs, very much conserved. For example, if you treat the antibody with proteolytic enzyme papain it has a new one side in each chain in the hinge region of the antibody. So, as a result, so far antibody molecules there are 2 sides, it will cleaved into 3 equal sized fragment one will contain the variable region and one constant region which is called Fab, F stands for fragment antigen binding.

So, if it cuts here there are 2 same size Fab is coming. So, there will in antibody there are what is the valency of antibody with respect to binding to antigen 2, so one antigen and one antibody molecule like this antibody molecule can bind 2 antigen in using 2 antigen binding site, but if you cut here, so, we each Fab will have only one antigen binding and if you see this F c stands for fragment crystallizable, these fragment is crystallizable.

Very careful why I am saying very careful because most of the time if we ask the question during viva or any other places what is F c stands for? It is fragments constant know, though it contain only constant domain but the F c stands for fragment crystallizable, no constant parties here. So, fragment antigen binding which can bind but it is a single valent, it can balance is one it can binds only one antigen and here it is F c fragment crystallizable.

So, neither Fab does not have the effector function, but effector function is mostly controlled by this Fc portion of the antibody, another enzyme called pepsin you see all these red arrows are the cut side. So it cut multiple sides but it is towards the C Terminus end of this disulfide part. So, as a result if you use pepsin in it breaks the antibody in several pieces, basically F c cut into multiple pieces, but Fab the fragment antigen binding is joined together because the disulfide bond is still there.

So, it named as Fab ab prime 2 because it has to Fab together. So it is valency is 2 just like antibody. After cutting with a proteolytic enzyme either pepsin or papain, they do not lose the antigen binding capacity, they can still bind antigen and many techniques or many kind of

technology during our research, we use either Fab or whole antibody or Fab prime. So have 2 prime and this is no use we cannot use this for anything, but it gives multiple pieces.

So, these 2 are biasing. So, they are that means, this proves that in general antibody molecule has some uniqueness, because the very few protein you will find like this if you take the antibody from any source whether mouse or human and you cut with papain we will get this result also it is highly this particular proteolytic cleavage sites are very much conserved and during this protein analysis the structure of antibody like presence of heavy chain, light chain, how many are there, how the link was the antibody structure basic structure antibody was no long before all this crystallization and recombinant technology is come in our hand.

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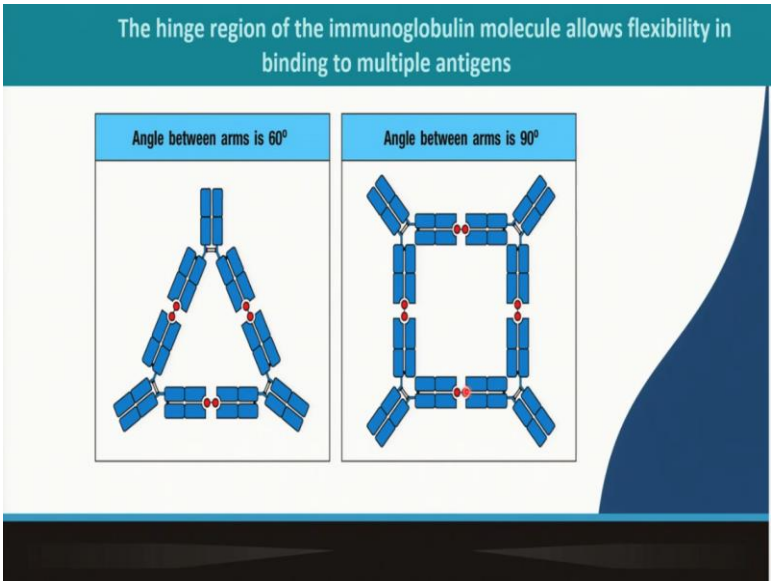

So, what this hinge region is doing hinge region in the antibody give I already told in the basic during the basic concept classes the hinge region gives them the flexibility. So, it is not rigid if it is fixed like that, it cannot go like this, it has to bind if one bind if this one these hand binds one antigen, it other antigen should be in this range it cannot go but here if it is flip between this side you had have more flexibility to bind antigen which you can see.

So, they see this kind of formation can happen. This is just a cartoon like this is the red is a hapten or small antigen which is too similar unit and if you give this hapten an antibody who makes these kind of triangular structure or this kind of square structure of 90 degree between

these 2 Fab and 2 Fab 90 degree can be there . So, this kind of formation is possible only because hinge region is there.

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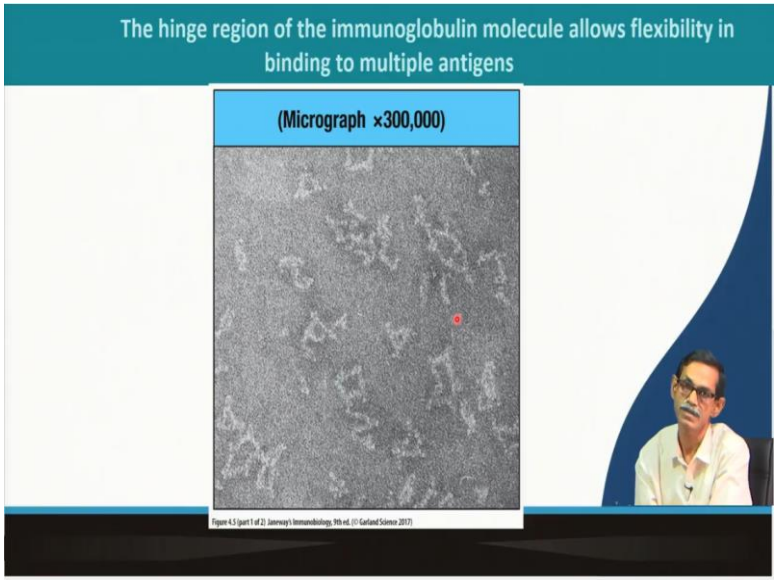

So, how do you know that this kind of formation is happening? This kind of formation is also can be seen in the electron micrograph you can see it is 300000 times magnified image if you see that there is a triangular formation. This is the same mixture like hapten repeat an antibody make sure if you incubate and give the take the image in electron micrograph. You can see a square like formation, you can see a triangular formation, you can see some straight lines means 2 antibody just one antigen 2 antibodies together, so 2 antibodies in straight line.

So, triangular and then square and linear all possible combinations are possible. So, this is telling that antibody hinge region makes antibody binding capacity more flexible and they can reach the antigen at different angle also, because it is not that one antigen binding site attraction other antigen binding sites remain idle or not doing anything. So, maximum possible way it can try and bind to the antigen.

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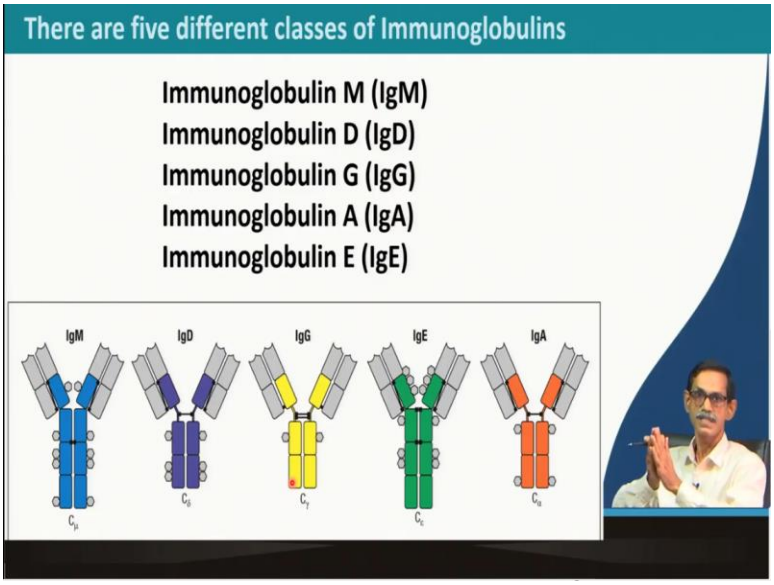

So, this is for your information. We will see again in different time point what they are doing, depending on the constant region of heavy chain antibody or immunoglobulin molecule can be divide into 5 subtypes, we call it Isotypes. Antibody molecule in human and in mouse we can divide into 5 Isotype, what are those? One is immunoglobulin M, immunoglobulin D, immunoglobulin G, immunoglobulin A and immunoglobulin E.

If you see it is the dividing in the cartoon in the bottom, you see there are different, we actually what we are discussing are telling you the structure or explaining the structure is a IgG. If you see carefully all these 5 slight difference you can see, let me start from right to left. Let me start here. So if you see IG compare IgA and IgG, you see they are very similar. The numbers of domains are similar.

They have hinge region, but the pattern of the disulfide bond it is this is the number here is 1 here is 2 and if you see the disulfide bond the location because disulfide bond form between 2 cysteine residues. So, this location of this disulfide bond is different. In this case, IgD are very similar to IgA, but why? What makes them different? Why they are different not only the structure actually what happened the amino acid sequences of these constant regions are different.

So, if you group them depending on the similarity in the amino acid sequence of constant region, you can find there are 5 types, and automatically there will be 5 subtypes and it was named Isotypes IgA, E, D, G, M. But this is not only their sequences different but the numbers of domains are also different. In this case of IgD, IgG and IgA the number of domains are same, but if you see IgM, and IgE, one domain they have extra.

They have 4 constant domains C H 1, C H 2, C H 3, C H 4 right which is not here, this is C H 1, 2, 3 here, so 4 domains, this is different. And if you see even more carefully both IgM and IgE they do not have the hinge region. So their flexibility is little less in comparison to IgG and IgA. What difference you can see? I can see something else, too. I can see the number of glycosylation, if you see this is not exactly representative of the number of you all these hexagons are the sugars, number of glycosylation, patterns are different.

They are not in the same location. Not only that IgE and IgM heavily glycosylated in comparison to IgG and IgA, the location is different even the variable region is also glycosylated which is not present anywhere else. So, this particular slide or the cartoon is selling 5 Isotypes IgM, D, G, A, and E are different in their constant domain. They are glycosylation are different. Their hinge is not present in all of them.

Glycosylation amount of glycosylation or degree of glycosylation is different between them although they are difference I mean they are function is very much different that we will see later. Like IgE is mostly responsible for allergy I am not telling all 5. Just to give you an example, IgE is mostly responsible for allergy. IgG is responsible for most of adaptive unit that we see and IgM is the first antibody that synthesized after an infection.

So, if you remember my basic concept class, the primary immune response and secondary immune response where I told that 2 points of difference one point was it primary response is more lag period or taking more time to initiate secondary responses quicker amount is different. And the third difference I am adding now or the third things that I am going to add now in that particular cord is the primary response, most of the antibody is IgM type.

Along with that point, one is I am increasing IgM is the antibody of primary response, but in secondary response, most of the antibody or antibody present in the secondary response are mostly IgG. So, this is so antibody structure very briefly I told you and these are the subtypes.

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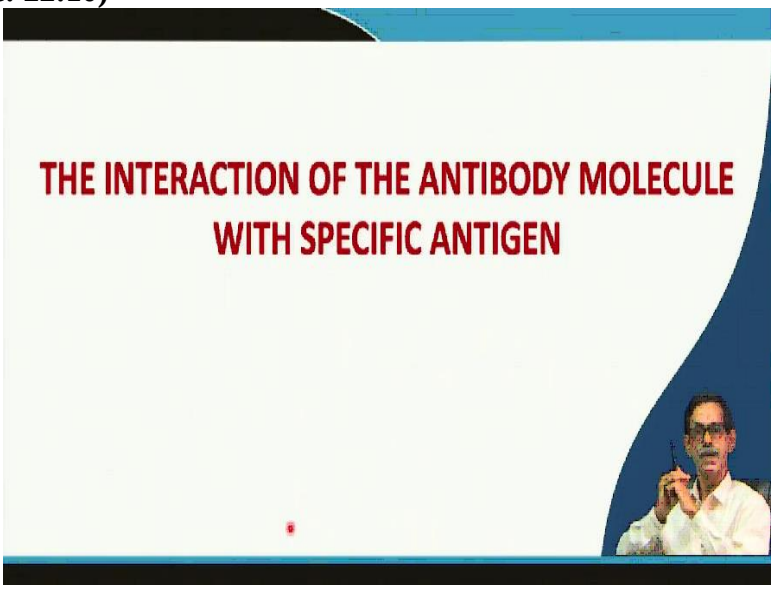

So, antibody molecule what the constant region is doing what the variable region is doing, what variable region which are all whole domain is variable, because do we need the whole variable domain to be different, in case of each different antibody, because if there are 5 different antigen, there should be 5 different antibody molecules, and variable region is responsible for interacting with antigen and whether all 5 different antibodies will have completely different variable region? No.

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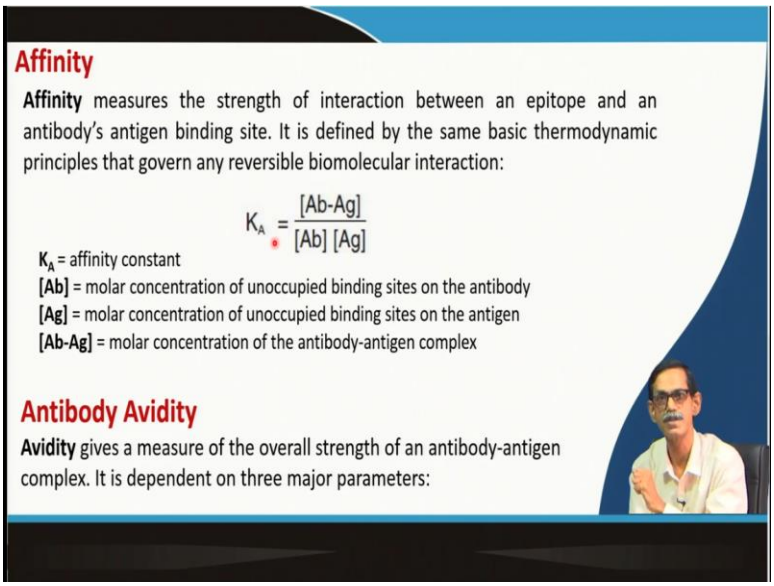

So, before that I let me introduce 2 more terminology. This is not exactly related to the antibody structure but I think, I mean this is a good time to introduce you because it may take you may find it many places and different times it is going to be very useful, one is Affinity, what is in general Affinity? Affinity between 2, proteins are; what Affinity between 2 proteins means they are special specificity in their interaction how good they interact.

More Affinity means there they will interact better in mathematically what we see that the major strength of interaction between an epitope you know what is epitope and antibodies antigen binding site. So, mathematically what is that the association constant is how many antigen antibodies are together that concentration divided by individual antibody concentration and antigen concentration. But I just telling you Ab is a short form or abbreviation of antibody and Ag is the short form of Antigen.

So, K A the association constant is the concentration of antigen antibody complex divided by Antibody, free antigen, free antibody and free antigen concentration. So, more the numerator value of numerator is more means better association. So, more number of antigen and antibody are associated together, more association is there that means, better the Affinity and as antibody has 2 valency. So, it is possible that one antibody can find only one antigen and it is also possible that one antigen find 2 antigen.

So, in that case antigen antibody complex maybe it is have any reversible it is a noncovalent interaction it is not a covalent interaction. So, what happens if this is the antigen antibody and if it binds one antigen here another antigen here. So, at any point time point if you see whether the antibody is completely free or antigen bound form, so maybe this is free, but this is still bound another time it binds but this is free.

So, what will happen? The total binding or the total binding of the antigen antibody or total binding of antibody towards the antigen maybe should not be compared with only one bound form. So, this term is called Avidity. One antigen binds together, so total binding will be more so, overall strength of antigen antibody complex depends on, what is the valency? What valency of antigen is there? Clear.

So this is antigen antibody interaction we normally say Affinity, higher Affinity antibody means high specificity antibody and high Avidity means when Avidity is very much important in some cases we may not need now during the discussion of antibody structure, but Avidity is important some other time. So I just introduced you the 2 terminologies if you have any question about the antibody, Affinity and Avidity there are enough number of I mean if you search net you will find it that what is this mean? So, and these Affinity or Avidity depend not Avidity the Affinity depends on where?

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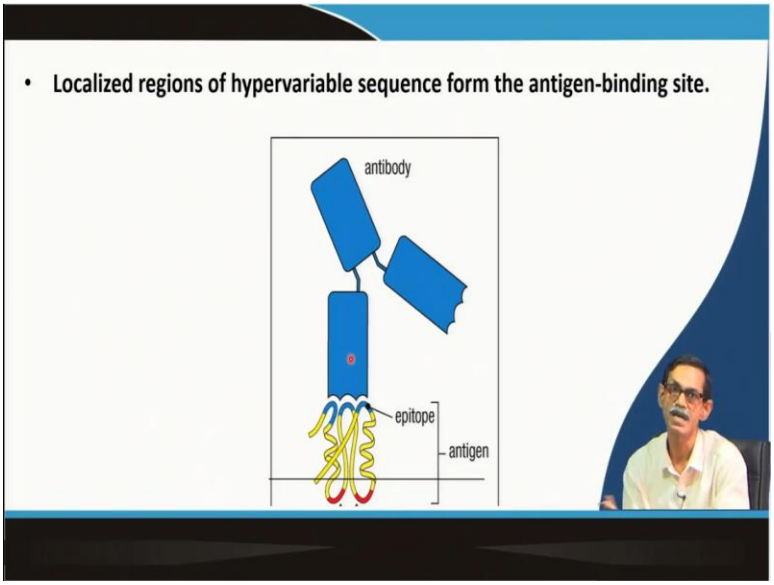

Affinity depends Affinity of antibody depends on Affinity of antibody depends on only this region if you see this picture we have seen before right the localized region of hyper variable sequence what happened actually, this is the whole antibody this is even a simpler form of drawing antibody, heavy chain light chain all merged together. So, this thing is half heavy chain and a half light chain right here.

So these part is a variable region and if you see carefully even within the variable region also not the whole variable region is interacting only the upper surface of the antibody, which contribute a very small part of the variable region actually interacting with epitope. So, if I have 10 different antibodies against 10 different antigens, if this region is different, then it is enough. We do not have to be different in different antibodies should not be different all variable region should be completely different.

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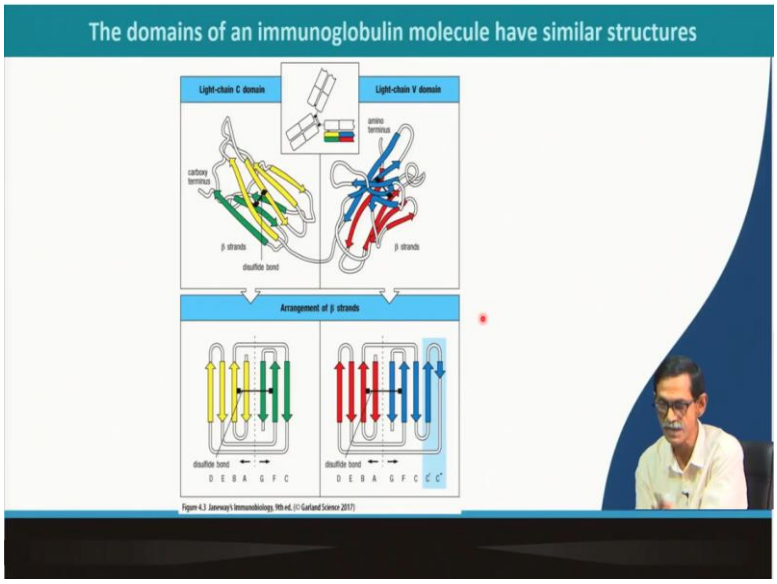

If I go to this part, you see most of the part is a structural part. It is basically the framework. So, a lot of beta strands out there, they are holding this they are basically doing like this structure and this upper surface is interacting with a this is the one domain if you see all the other fingers and pump are the antibody, I mean the beta stand part. So, this upper part is doing this and this loop. So, this form is just like a loop.

So, these loops are the exposed part these strands are making just a framework or these maintaining the structure of the protein in that part and this loop which is exposed. So, these loop which we will see later, that these loops are the only place which is interacting with antigen. So, if you see this 3 loop have epitope antigen and this is the part of antibody which is interacting which is nothing but all this loops what we see in that domain structure.

So, these finally, the loop of the outer loop of the antibody and epitope these 2 are interacting and these 2 interaction, how good it is depending on that how specific and how what is better Affinity or lower Affinity will depend on that it is a noncovalent interaction and it follows all the rules of protein-protein interaction because most of the cases antigens are protein an antibody definitely by now you know it is protein. So, how these, what is the structure of these Loop region and how it varies, which part it is, how big it is, what is their length? We will discuss in the next class. Thank you.

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