Fundamentals of Protein Chemistry
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Module - 10
Protein Macromolecule Interactions II
Lecture - 47
Protein Carbohydrate Interactions - II

In our second lecture on protein-carbohydrate interactions, we will be looking at the specifics of these types of interactions in a bit more detail.

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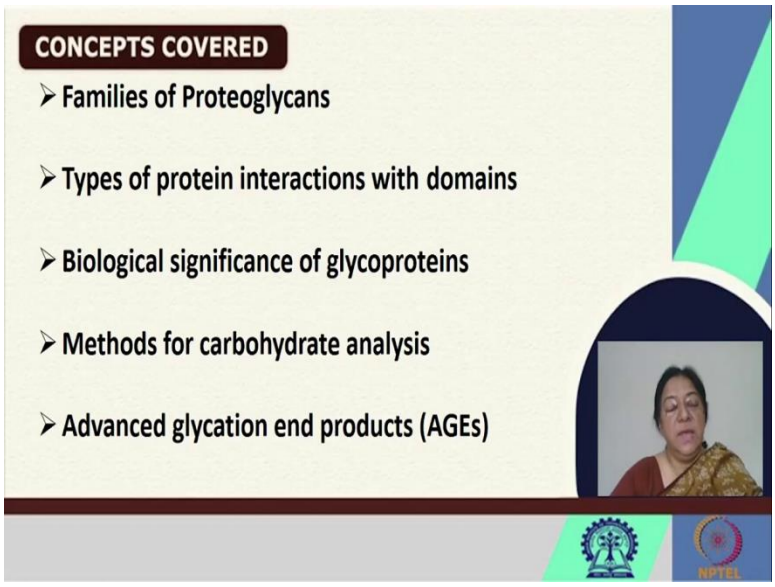

Here we will be looking at families of proteoglycans, the types of protein interactions with the specific carbohydrate recognition domains and what the biological significance of these glycoproteins are, methods for analysis and what we mean by advanced glycation end products.

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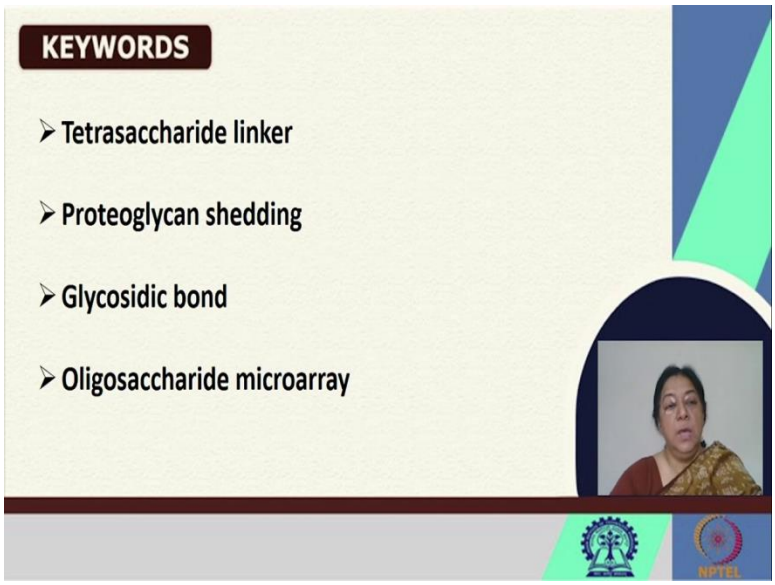

In a tetrasaccharide linker that we have for the proteoglycans, we will see what amino acid residues are involved in this specific recognition.

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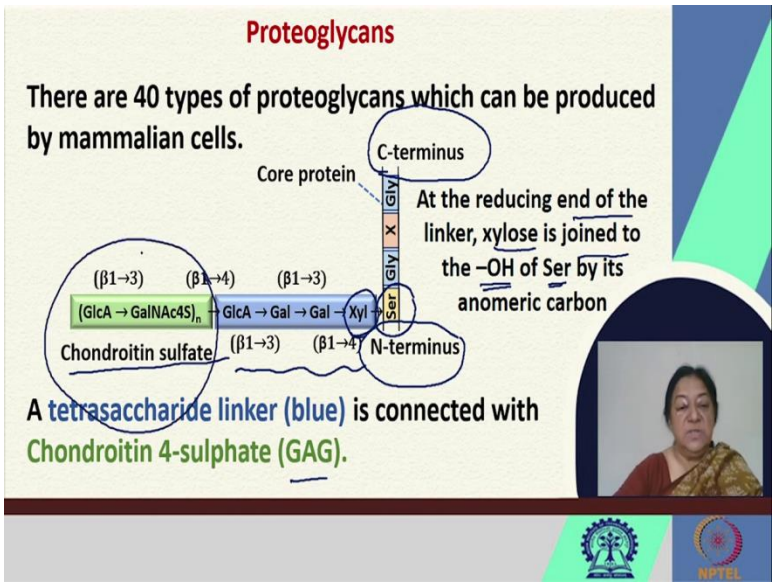

If we look at what proteoglycans are, there are around 40 types of proteoglycans which can be produced by mammalian cells. Here [refer to slide], there is a core protein that has a C-terminus and an N-terminus. What happens is, we have a tetrasaccharide linker that is blue and is connected with the chondroitin 4-sulphate.

We looked at the specific type of linkers that are available, where we have the core protein and we remember that it is linked to the serene amino acid residual. Now at the reducing end of the linker here, xylose for example in this particular case, is joined to the OH of the serine amino

acid side chain by its anomeric carbon and this tetrasaccharide linker, then connects the protein with this specific chondroitin sulfate.

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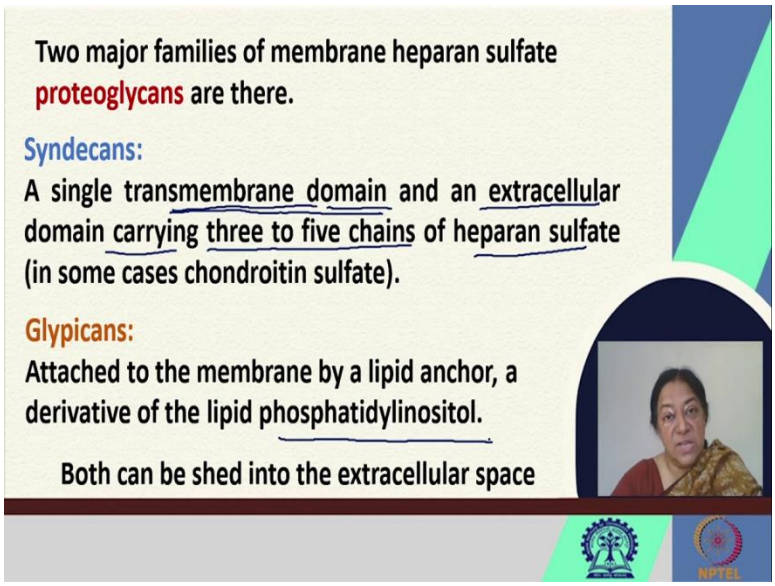

There are two major families of membrane heparan sulfate proteoglycans. One are the syndecans. This is a single transmembrane domain. When we looked at the membrane proteins, we found that they were based on carbohydrate linkers to these proteins, that were available for several biological activities including cell-cell adhesion, signal transduction and so on so forth.

To understand how these are linked together it is important to look at their domains, their specific types of interactions and how they may be linked to other proteins. This specific type, the syndecan is a single transmembrane domain and it has an extracellular domain that carries three to five chains of a heparan sulfate. In some cases, this may be also chondroitin sulfate.

Glypicans on the other hand, are attached to the membrane by a lipid anchor, a derivative of the lipid phosphatidylinositol. We have the linkages here of the carbohydrates to the protein, that can be involved by a lipid anchor or could have a specific domain that would link these to the proteins. Now, what can happen is both of these can actually be shared to the extracellular space available.

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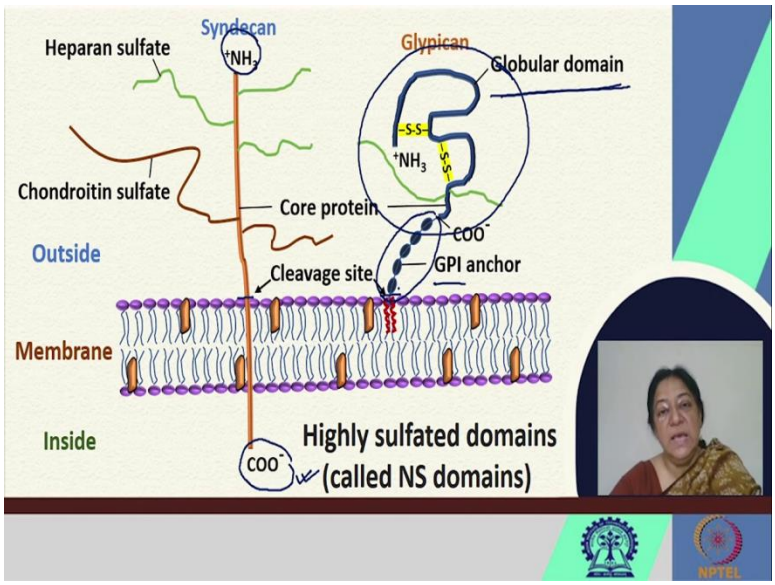

There are specific domains that are called highly sulfated domains. So if we look at a pictorial representation of our membrane, a lipid bilayer membrane and try to identify and look at the different connectivities that link these carbohydrate units to our proteins of interest.

So, if this [refer to slide] is a cartoon representation of the protein chain; we have the amino and the carboxy terminals and to this we have attached say heparan sulfate or chondroitin sulfate on the outside. This then would be recognized by other proteins or by other activities in the cell, other the moieties in the cell, that would link these with them for the specific functionalities that would be required.

In this case, we have a glyco anchor here, the GPI anchor and we have a globular domain. This is the linker and this is our domain which has as we can see disulfide linkage, a regular protein domain. The core protein that is present here is then linked to the sites. For there are sometimes specific cleavage sites that shed these glycoproteins to the extracellular space. This is an example of syndecan and this is an example of a glypican.

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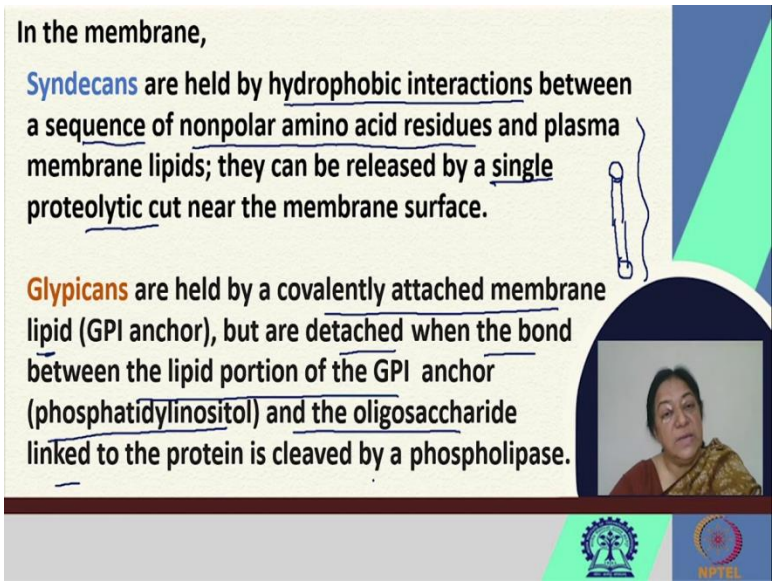

In the membrane therefore, we have the syndecans that are held by hydrophobic interactions because they were in the lipid membrane bilayer that we saw. So, we know that the lipid membrane bilayer and the protein was a single transmembrane protein. The hydrophobic interactions are important here, where we would have a sequence of nonpolar amino acid residues and plasma membrane lipids.

What happens is, they can be released by a single proteolytic cut near the membrane surface, as we saw in the previous slide. Glypicans on the other hand are held by a covalently attached membrane lipid, that is the GPI anchor and they can be detached where the bond between the lipid portion of the GPI anchor, that is the phosphatidylinositol and the oligosaccharide that is linked to the protein, is cleaved by an enzyme called phospholipase. This is where the specific glycoprotein can be released due to a proteolytic cut, that is enzyme driven.

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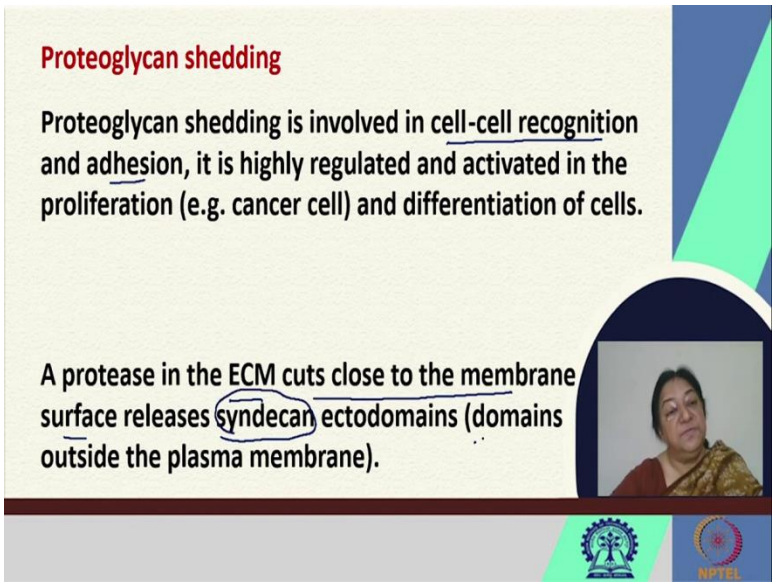

This proteoglycan shedding is involved in cell-cell recognition and adhesion and is highly regulated and activated in the proliferation and differentiation of cells. So, a protease in this extracellular matrix, cuts close to the membrane surface and it releases these ectodomains of the specific proteoglycans.

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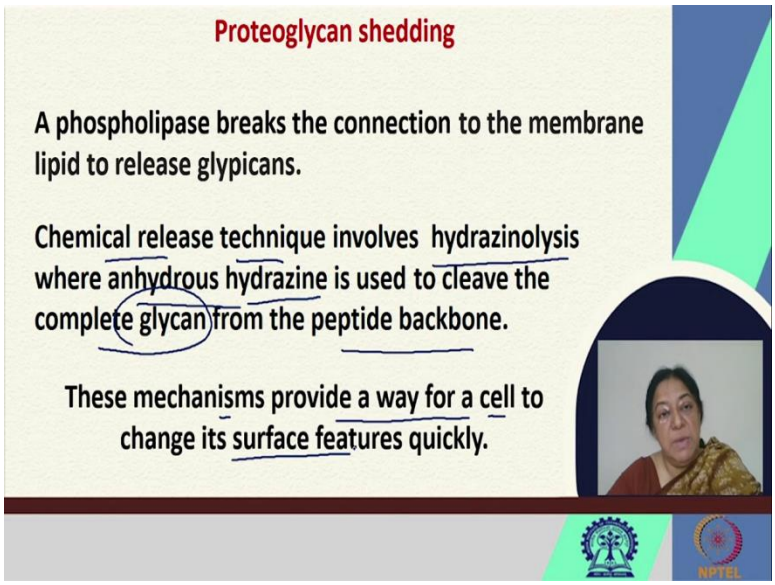

The proteoglycan shedding for example has this phospholipase, the enzyme that breaks the connection to the membrane lipid to release the glyphicans. We can also have chemical release of the high glypicans, where in this case what happens; there is hydrazinolysis where anhydrous hydrazine is used to cleave the complete glycan from the peptide backbone.

So, we understand that this is necessary for the proteoglycan shedding for specific activities related to the cell. Now what happens is, these mechanisms that result in proteoglycan shedding, are a very common way for a cell can change its surface features quickly.

The integral cell membranes have carbohydrate connections to them and they are cleaved off. Due to this the membrane surface will adapt to a different type of surface feature and this may be involved in surface-surface recognition for cell-cell adhesion and other activities as well.

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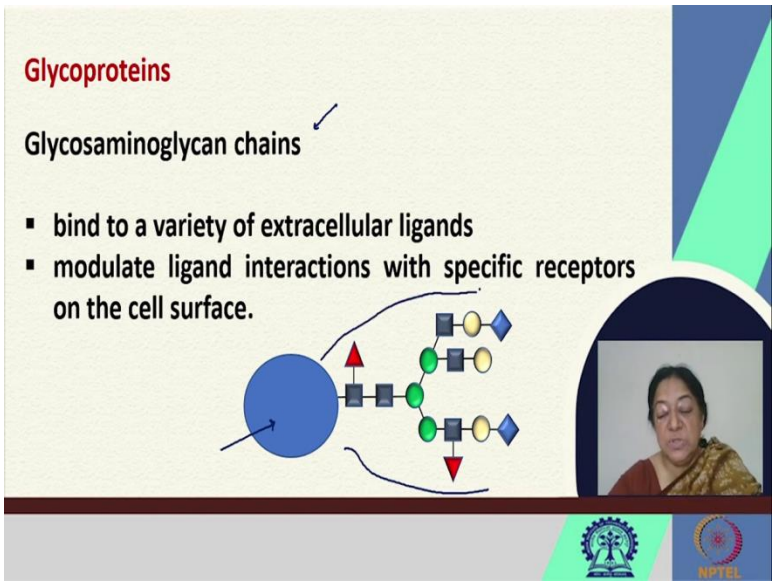

The glycoproteins in general, are specific glycosaminoglycan chains. They bind to a variety of extracellular ligands and they can modulate the ligand interaction with specific receptors on the cell surface, which are important for the functionalities that they are involved in.

If this [refer to slide] is our overall protein chain that is either embedded or on the surface of the cell membrane, then we can have a variety of linkages of these different types of carbohydrates that can then be used for different properties.

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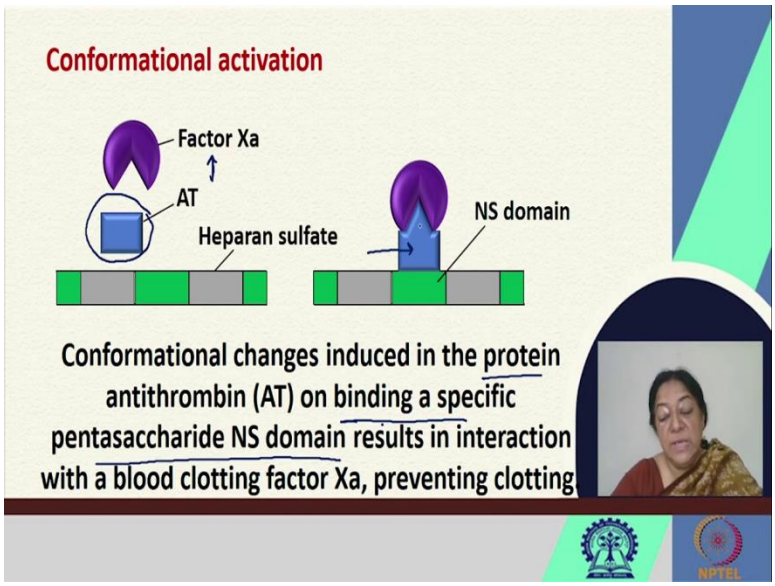

For example, if we look at a conformational activation; a conformational activation where we have heparan sulfate and we have the antithrombin. So there is a protein antithrombin on binding a specific pentasaccharide NS domain, that results in the interaction with a blood clotting factor.

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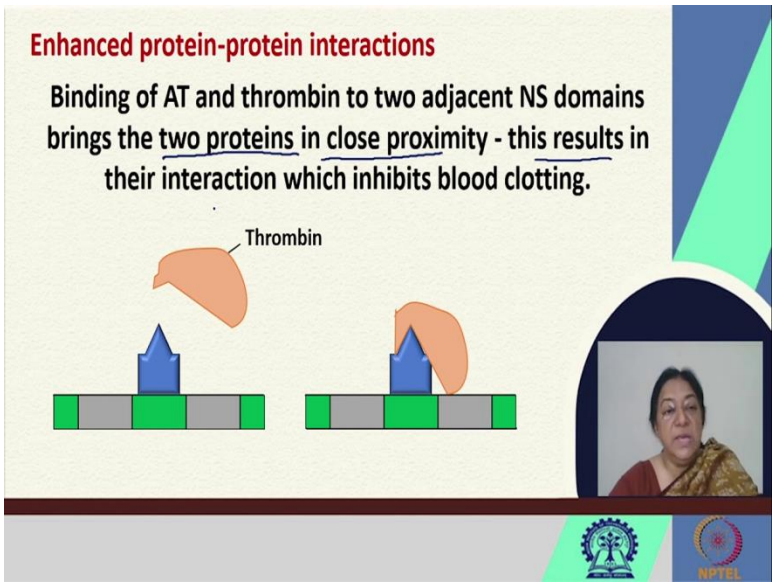

This blood clotting factor prevents the blood clotting. So what happens in this case is, the AT binds to the surface, it changes its surface functionality, changes the characteristics of its surface because it has now bound the pentasaccharide to it.

Due to this structural feature, the factor Xa, which is a blood clotting factor, can then be attached to the antithrombin. This means that there are enhanced protein-protein interactions possible because of these carbohydrate variations.

If we look [refer to slide] at a specific site here, where we have the modified antithrombin and then we have the thrombin come into the picture. If this binds, then the binding of the antithrombin and the thrombin will occur to two adjacent NS domains and bring the two proteins in close proximity. This results in the interaction, which inhibits blood clotting.

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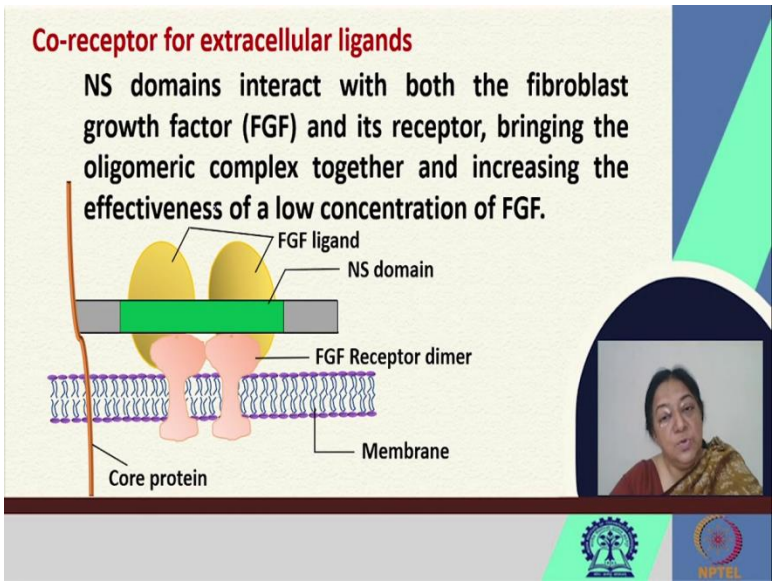

So, this is the way the specific workings of the cell occur due to the protein-protein interactions, based on carbohydrate binding. We have the co-receptor for extracellular ligands as well. Here, the NS domains interact with both the fibroblast growth factor and its receptor and what happens in this case, it brings the oligomeric complex close together and increases the effectiveness even at low concentrations of the growth factor.

If we have our lipid membrane, we can see that the important aspects here are the specific proteins that are bound to the lipids, where we have most of the carbohydrates bound. We have our core protein that traverses the membrane and we have specific receptor proteins in the membrane. This [refer to slide] is an FGF receptor dimer, that is a fibroblast growth factor dimer and a specific domain, that then allows the binding of the FGF ligand which is useful for its specific activity.

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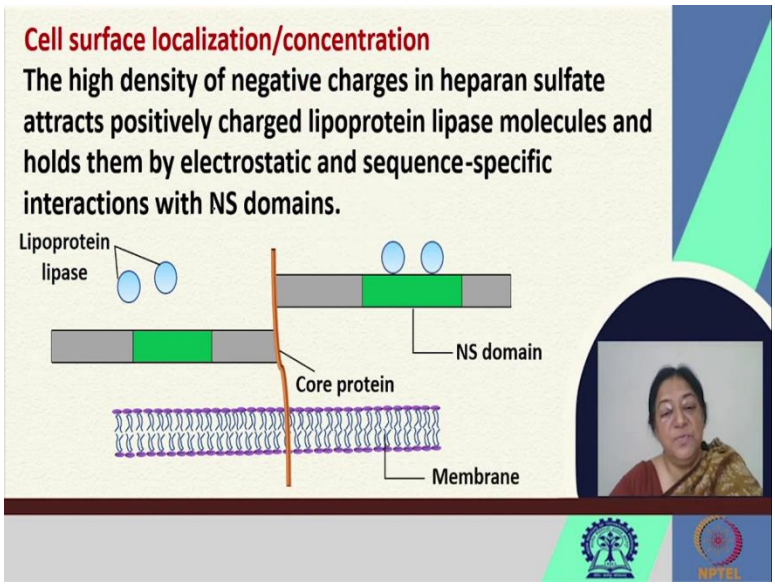

This means that the cell surface localization and concentration is important and the interesting aspect is the high density of the negative charges in the heparan sulfate, that can attract the positively charged lipoprotein lipase molecules and hold them in place non-covalently, by electrostatic and sequence-specific interactions.

We have a complementarity in the chemical moieties here, where there is a possibility of specific non-covalent interactions. We have our protein embedded here [refer to slide], specific domains from the core protein that would be able to have the lipoprotein lipase attack to it and as a result of which, we would have the cleavage or we would have a specific activity associated with the lipoprotein and the advantage of having the complementarity in charges, allows this connection to take place.

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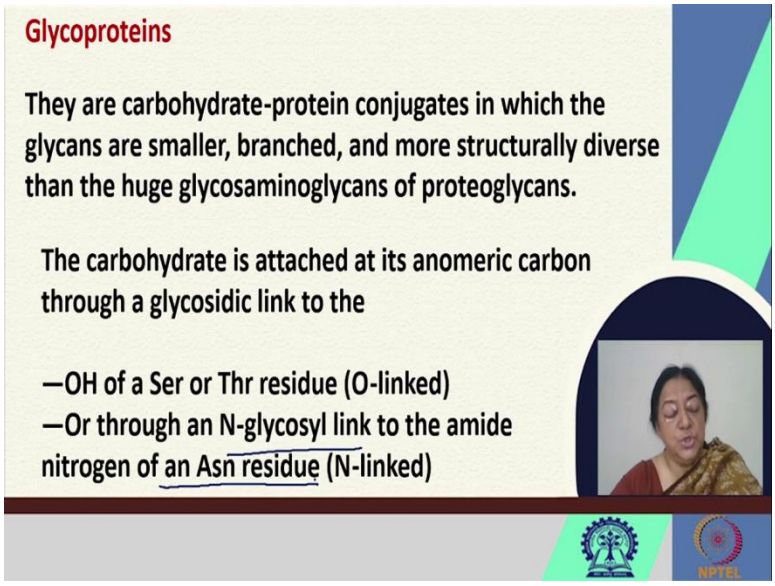

In glycoproteins, the carbohydrate-protein conjugates; the glycans are smaller, branched and they are more structurally diverse than the other types of larger molecules that we saw, like the glycosaminoglycans of the proteoglycans. So, these glycoproteins are relatively smaller in size.

The carbohydrate in this case is attached to its anomeric carbon through a glycosidic link. This is either to the OH of a serine or a threonine residue (which we saw in the previous lecture) or through an N-glycosyl link, to the amide nitrogen of an asparagine residue. So, these residues are important in the connectivity of the protein with the specific sugar moieties.

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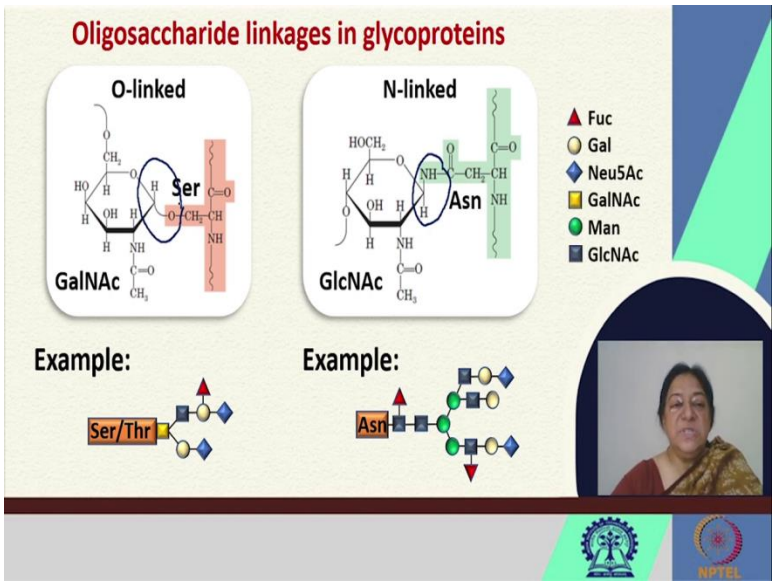

In the oligosaccharide linkages in glycoproteins, we have O-linked type, where we have the serine residue that is linked to a sugar or we have the N-linked type, where we have the asparagines So here [refer to slide] is our N-linked type and here is our O-linked type, depending upon the specific linkages with the specific types of ligands, based on the amino acid residues in this case serine and asparagine.

If we look at specific examples this is the way they would be connected in their oligosaccharide linkages in the glycoproteins. So when we look at these specific types of linkages, we realize their importance.

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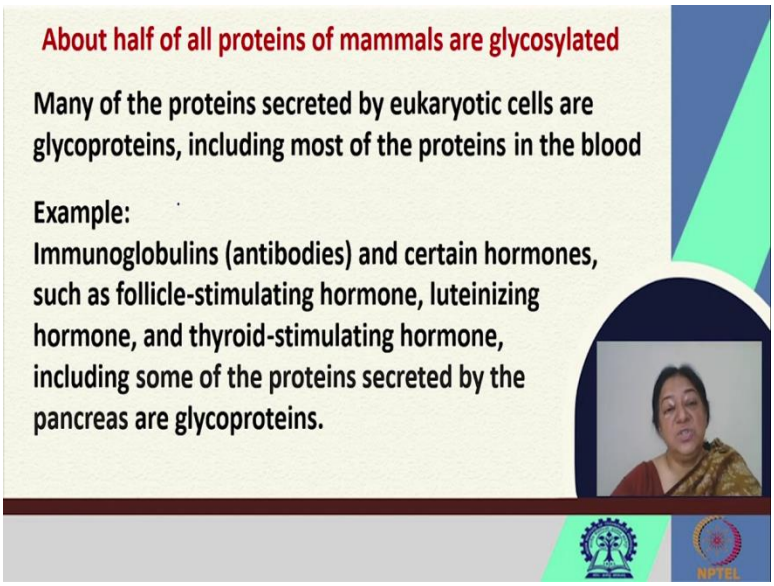

About half of all proteins of mammals are actually glycosylated and many of the proteins secreted by the eukaryotic cells are glycoproteins, including most of the proteins in the blood.

For example, immunoglobulins and several hormones, such as the follicle-stimulating hormone, the luteinizing hormone, the thyroid-stimulating hormone and some proteins that are secreted by the pancreas. All of these are glycoproteins. Many of them have their functionalities based on the carbohydrate that is linked to the protein.

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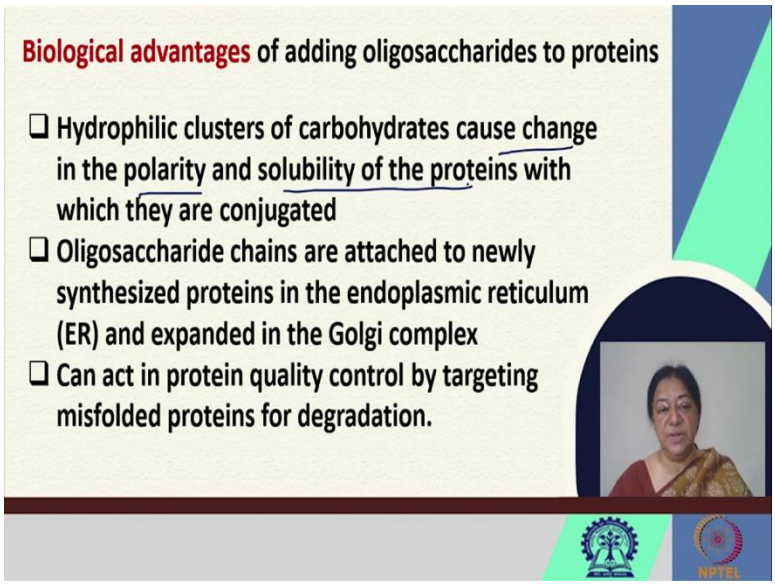

We now look into the biological advantages of adding oligosaccharides to proteins. The adhesion of these hydrophilic clusters of carbohydrates, can often change the polarity and the solubility of the proteins with which they are conjugated. As a result, the oligosaccharide chains can be attached to newly synthesized proteins in the endoplasmic reticulum and expanded in the Golgi complex. They can also act in protein quality control, by targeting misfolded proteins for degradation.

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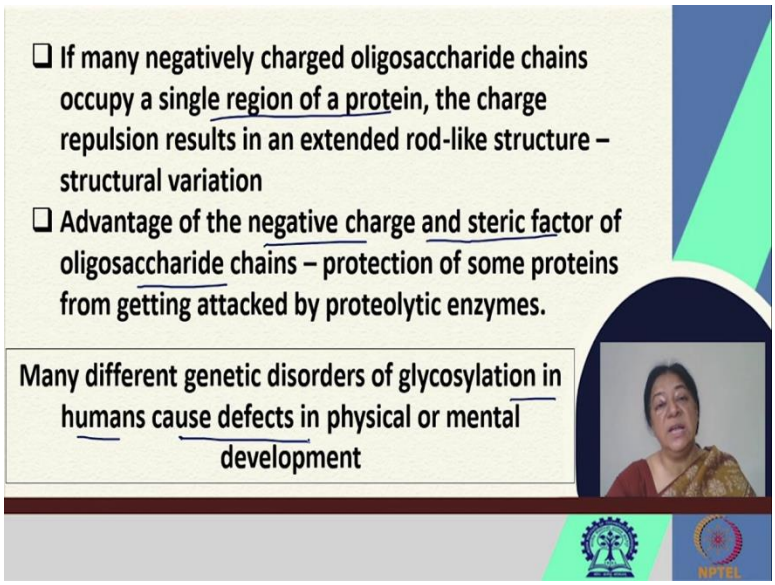

The other ways in which they could be advantageous are, if there were many negatively charged oligosaccharide chains that occupy say a single region of the protein, then the charge repulsion could result in structural variation or conformational changes in a way, that would result in an extended rod-like structure that may be important to the proteins functionalities.

The advantage of the negative and the steric oligosaccharide chains, sometimes give protection to some proteins from being attacked by proteolytic enzymes. There are also many different genetic disorders of glycosylation, that are present in humans that actually cause defects in physical or mental development. So we understand the advantage of having the oligosaccharide chains attached to our proteins.

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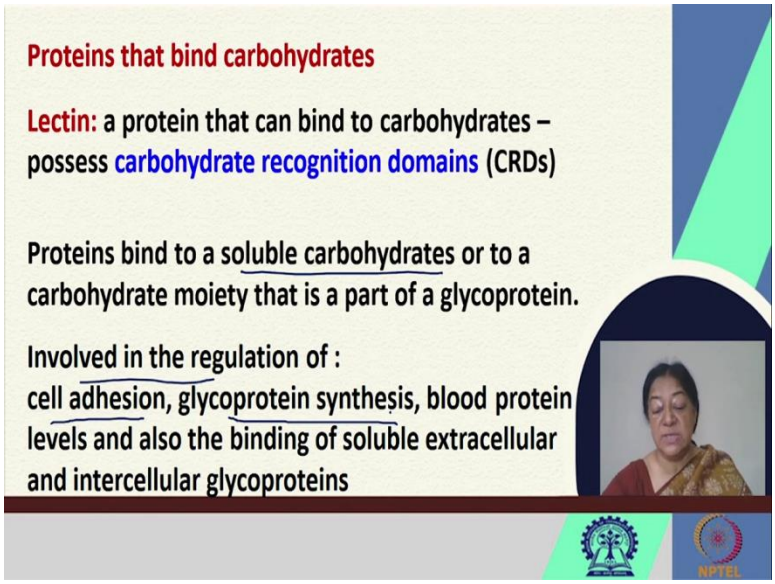

The proteins that bind carbohydrates is something that we saw in the previous lecture, where we looked at lectins. This lectin is a protein that can bind to our carbohydrate, but in most of these cases we find that the carbohydrates are linked directly to the protein molecules.

However, these lectins can look at specific soluble carbohydrates and they can bind to the soluble carbohydrates individually or bind to a carbohydrate moiety that is part of a glycoprotein. So we could have a conjugation in a manner, that is commonly seen on membrane proteins. This is involved in the regulation of cell adhesion, in glycoprotein synthesis, to regulate blood protein levels and also in binding of soluble extracellular and intracellular glycoproteins.

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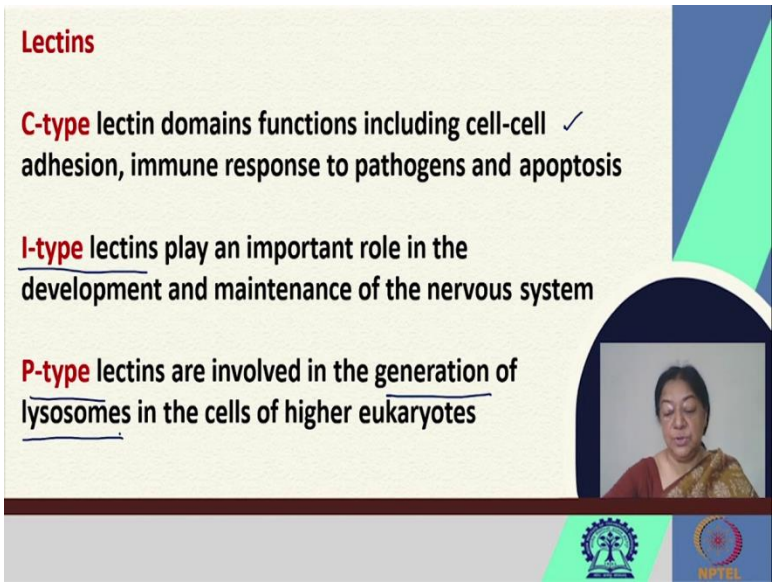

The three different type of lectins that we looked at in the previous lecture, were the C-type, the I-type and the P-type lectins. So, each of these lectins have a carbohydrate recognition domain. The lectin domains in the C-type play a role in cell-cell adhesion, immune response to pathogens as well as apoptosis.

The I-type lectins play an important role in the development and the maintenance of the nervous system and the P-type lectins are involved in the generation of lysosomes in the cells of higher eukaryotes. The lectins have very high binding affinities for specific sugars, as would be expected.

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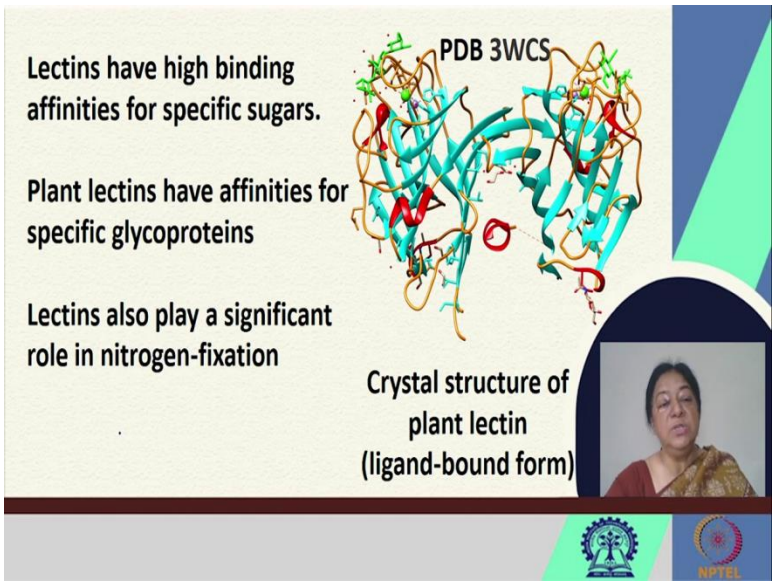

For example, plant lectins also have affinities for specific glycoproteins and in the process, they play a very significant role in nitrogen-fixation. This [refer to slide] is an example of a crystal structure of a plant lectin in its ligand-bound form. So, the importance of lectin in their affinity for specific sugars, involves an understanding of their specific functionalities.

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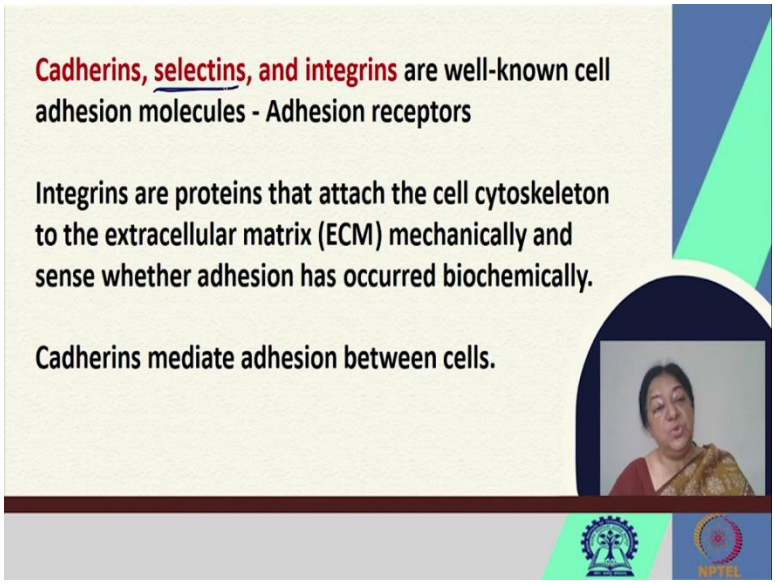

Other types of proteins that are also known as lectins are the cadherin, selectins, and integrins; all of these are well known cell adhesion molecules, as adhesion receptors. The integrins are the proteins that attach the cell cytoskeleton to the extracellular matrix mechanically and then they sense whether the adhesion has actually occurred through biochemical manner. The cadherins on the other hand, mediate the adhesion between cells.

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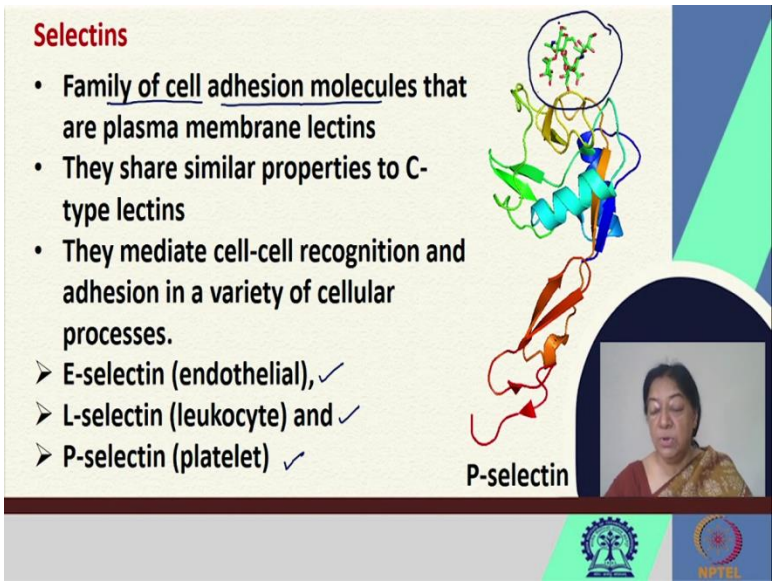

There are different types of selectins based on where they attach to. So, this is also a family of cell adhesion molecules, that are plasma membrane lectins. They share similar properties with the C-type lectins, because they are involved in cell-cell adhesion.

They mediate cell-cell recognition and adhesion in a variety of cellular processes. There is the E-selectin that is the endothelial cell selectin, then there is the leukocyte cell selectin that is the L-selectin and the P-selectin a platelet like selectin. So, this [refer to slide] is a structure of the P-selectin molecule and we can see the bound portion of the carbohydrate here.

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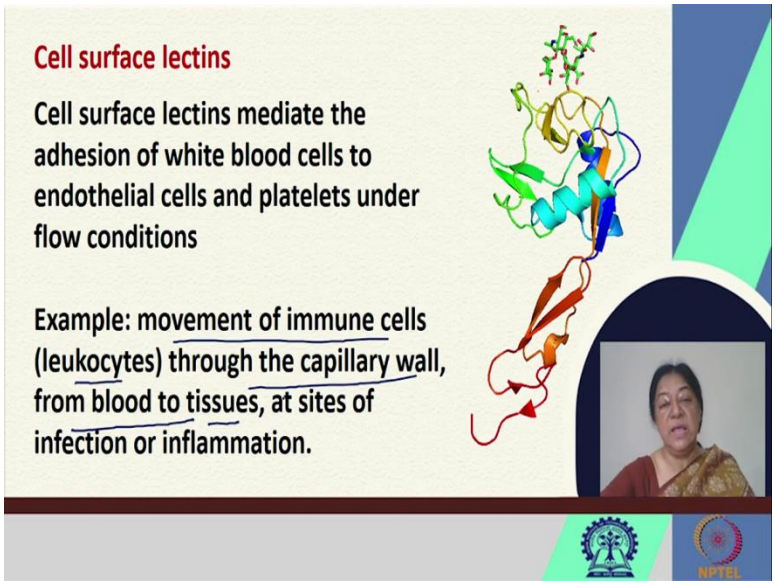

So if we look at these cell surface lectins, their importance lie in the adhesion of the molecules. For example, cell surface lectins can mediate the adhesion of white blood cells to endothelial cells and platelets under flow conditions.

For example, we can have the movement of immune cells the leukocytes through the capillary wall, from blood to tissues, at the sites of infection or inflammation and these lectins are involved in the cell adhesion properties or the cell adhesion functionalities, that are required for this process to occur.

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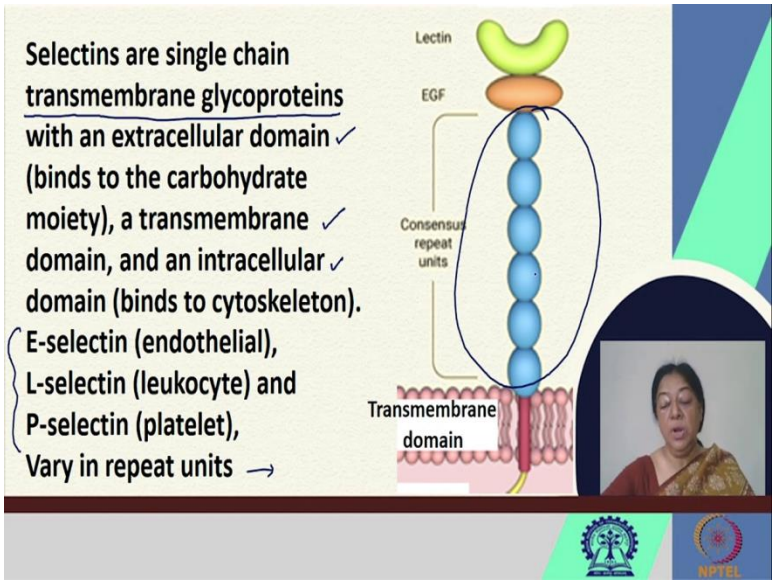

The selectins are single chain transmembrane glycoproteins. We had discussed transmembrane glycoproteins in our membrane proteins lecture. What they have is, they have an extracellular domain that binds to the carbohydrate moiety, a transmembrane domain and an intracellular domain that binds to the cytoskeleton.

So, this [refer to slide] is the basic structure of lectin and in the variety of lectins that we saw; the E, the L and the P, what they do is they have a variation in their units for this. We have the E-selectin, the P-selectin, and the L-selectin and each of these have a variation and it could be 3, 5, 7, 9 units, depending upon their specific function.

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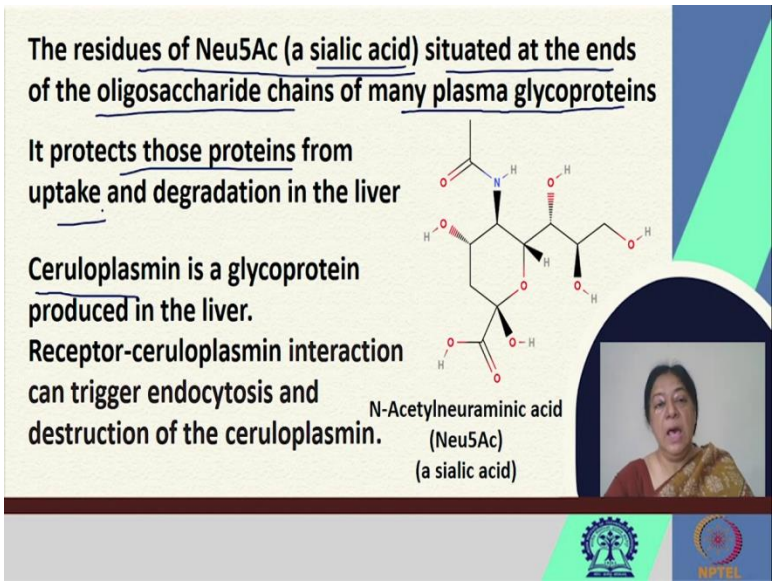

The protection that we see are residues of a sialic acid that are situated at the ends of oligosaccharide chains of many plasma glycoproteins. Now what these do is, these protect the proteins from uptake and degradation in the liver.

For example, this is like a protective cap. Now if we look at ceruloplasmin, this is a glycoprotein that is produced in the liver. The receptor-ceruloplasmin interaction can trigger endocytosis and could result in the destruction of ceruloplasmin. However what happens, the residues of sialic acid can protect these proteins from uptake and degradation in the liver.

So, we looked at these different types of oligosaccharides that can be attached to the proteins and also a methodology of lectins that bind to the carbohydrates themselves. We did not go into the basic mechanism of action, but understood the role of these proteins in cell-cell adhesion and other properties associated with the cells and their biochemical processes.

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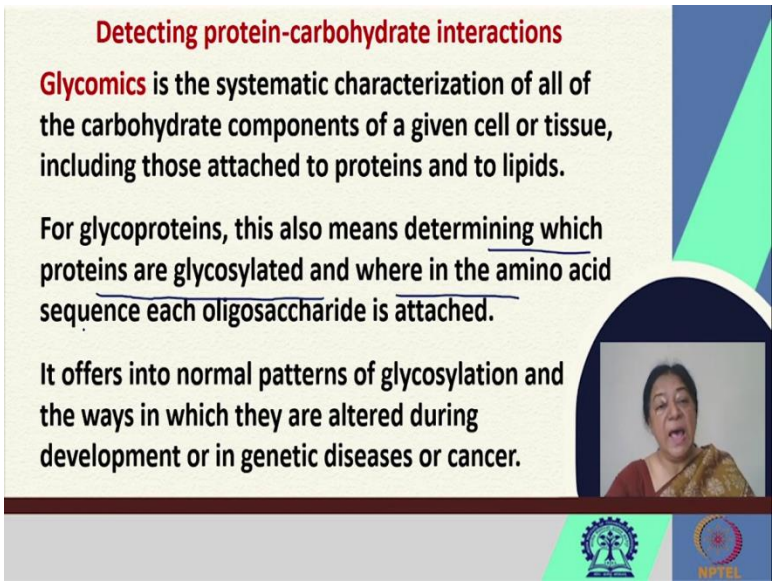

But we would also need to know how we can detect protein-carbohydrate interactions and in this case, the glycomics is the systematic characterization of all of the carbohydrate components of a given cell or tissue, including those that are attached to proteins and to lipids. So, what happens if we do a test for glycoproteins, it can determine whether the proteins are glycosylated and where in the amino acid sequence the oligosaccharide is attached. It also offers normal patterns of glycosylation and can be altered in the case of disease.

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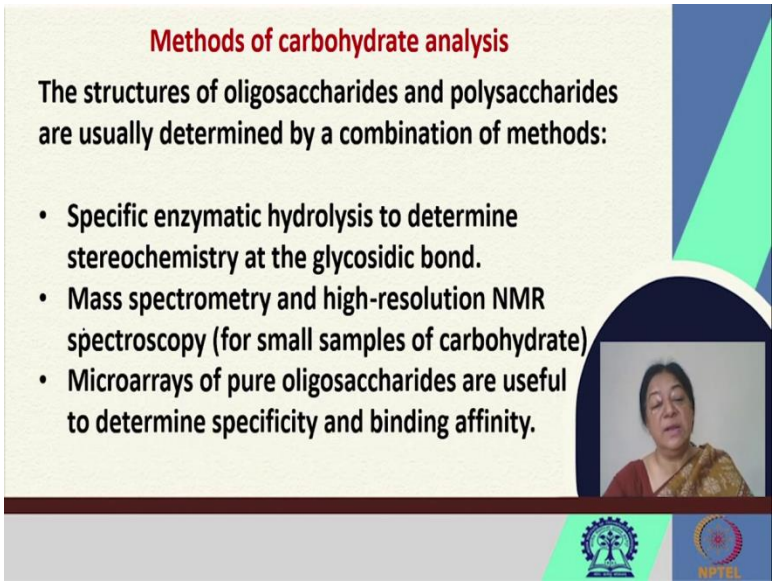

So if we look at the methods of carbohydrate analysis, the structures of oligosaccharides and polysaccharides are usually determined by a combination of methods. There is a specific enzymatic hydrolysis to determine the stereochemistry of the glycosidic bond.

There is mass spectrometry and high-resolution NMR that can be used for small samples of carbohydrates. In adhesion, there can be microarrays of pure oligosaccharides that are useful to determine specificity and binding affinity.

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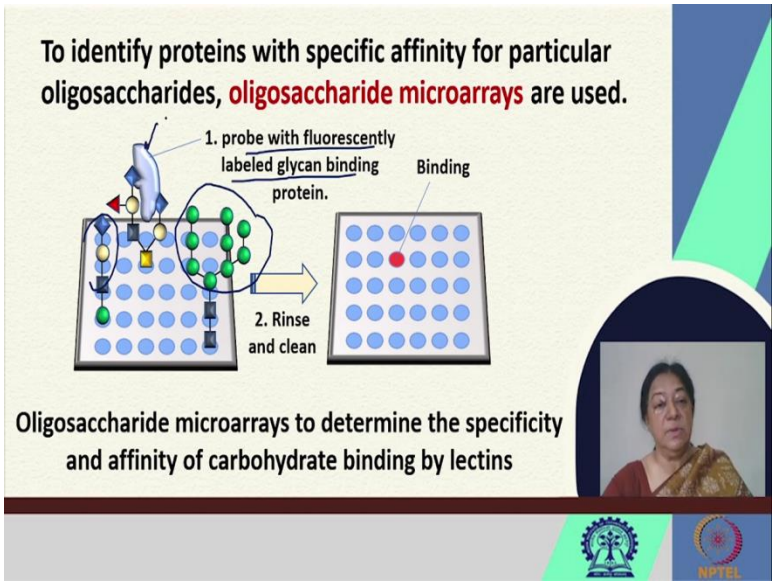

For example, if we look at a microarray. To identify proteins with specific affinity for a particular oligosaccharide, we can use an oligosaccharide microarray. This [refer to slide] is a cartoon representation of microarray plate and we have the specific oligosaccharide attached to this microarray plate. This is then flushed with protein and the specific protein may bind to one of the oligosaccharides that are attached to the microarray. We have the probe with the fluorescently labeled glycan binding protein. Then we rinse and clean.

Those that have a binding protein attached to the oligosaccharide would remain and they can be detected. So, there will be binding and they can determine the specificity and binding of the carbohydrates, because we see all these different moieties that did not have a specific attachment site for the protein under determination.

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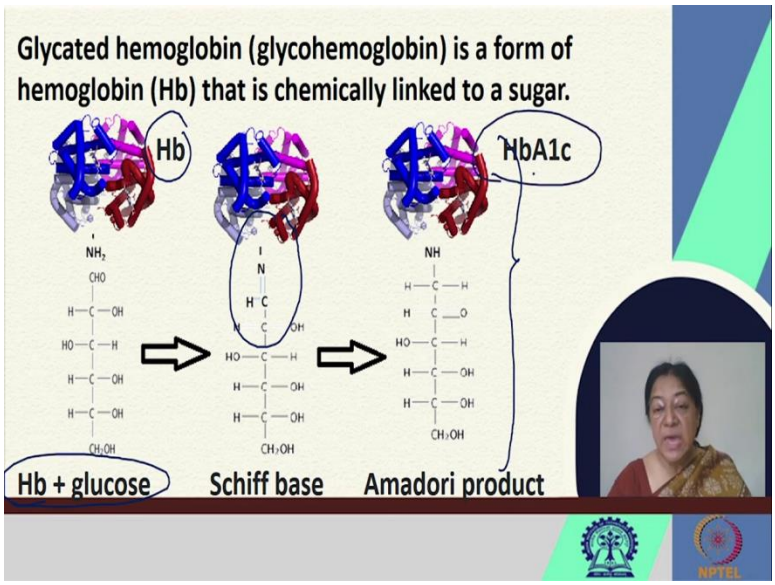

Another very important method is glycated hemoglobin detection, which is a form of hemoglobin that is chemically linked to a sugar. In this case, we have the hemoglobin plus the glucose that forms what is called a Schiff base.

This Schiff base can form what is called an Amadori product that can be detected by a specific chemical method. This is called Hb the hemoglobin and once it is attached, it is HbA1c. This is a typical test method to determine the amount of sugar connected.

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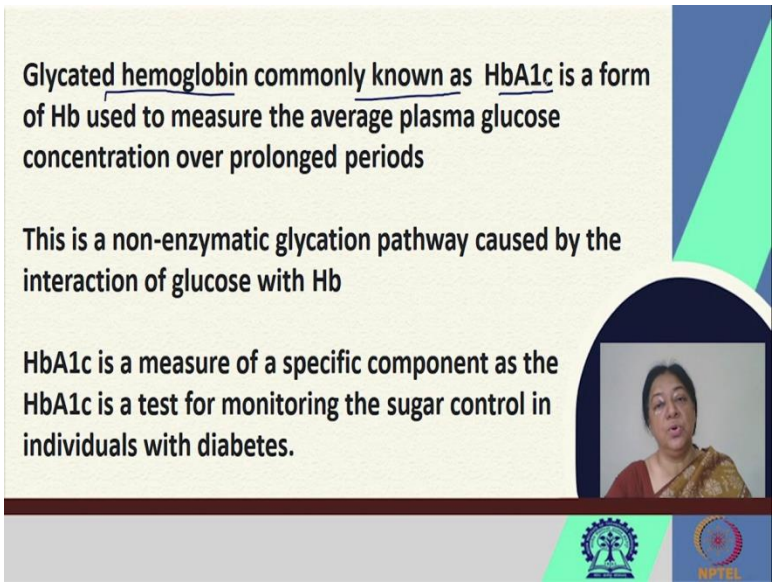

So glycated hemoglobin commonly known as HbA1c, is a form of hemoglobin that is used to measure average plasma glucose concentration over prolonged periods and is a commonly used pathological test. It is a non-enzymatic glycation pathway caused by the interaction of glucose

with hemoglobin and HbA1c is a measure of a specific component which is a test for monitoring the sugar control, specifically in individuals with diabetes.

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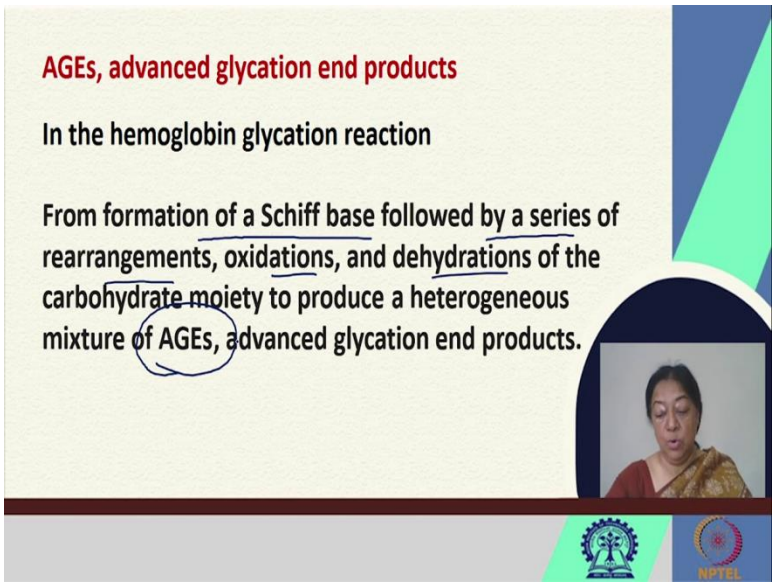

In the hemoglobin glycation reaction, there are the production of advanced glycation end products. We looked at the formation of the Schiff base, followed by a series of rearrangements, oxidations and dehydrations. A lot of chemical processes, biochemical processes are involved and it produces a mixture of what are called advanced glycation end products or AGEs, that can be monitored to check the extent of the glycation reaction on hemoglobin.

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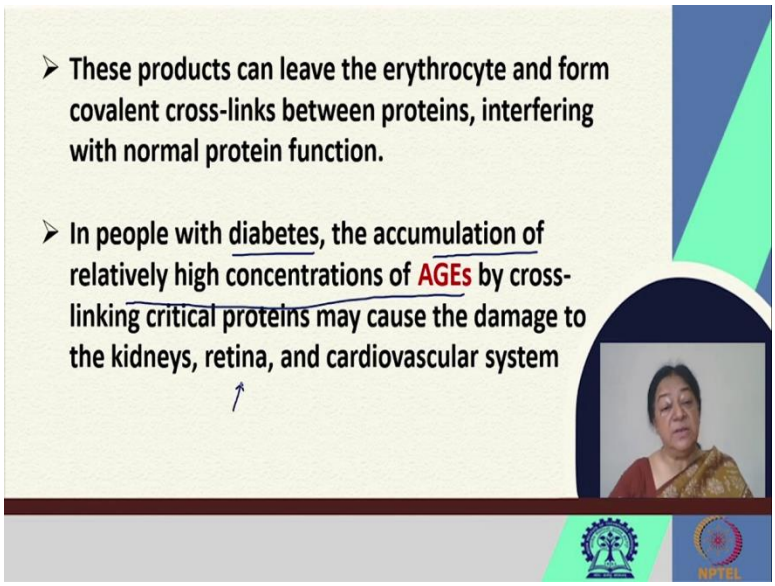

These products can leave the erythrocyte and form covalent cross-links between proteins and what they will do in that case, they would interfere with normal protein function. So, in people with diabetes, there is the accumulation of the high concentrations of AGEs, the advanced glycation end products by cross-linking critical proteins that can cause damage to the kidneys, the retina and also the cardiovascular system. You probably heard about diabetic retinopathy, which is where the retina is involved.

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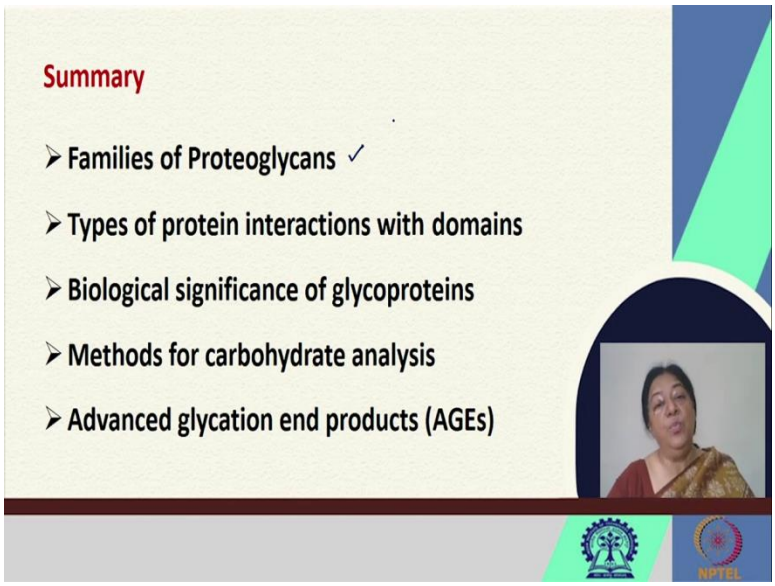

So what we have looked at in these two lectures of proteins and carbohydrate interactions, is we have looked at the family of proteoglycans, the types of protein interactions with domains, which amino acids are specifically involved in the interaction with the carbohydrates, the biological significance of the glycoproteins and methods for carbohydrate analysis.

An example of how we have the lectins that are the specific proteins that bind carbohydrates or carbo glycoproteins, where they have already been bound to a sugar and we looked at advanced glycation end products, in a manner to check for diabetic patients in the case where the glucose levels are high.

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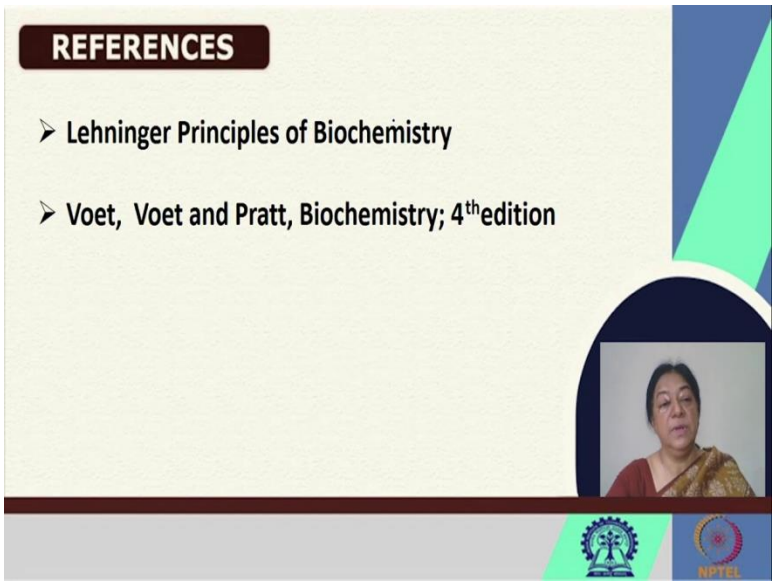

These [refer to slide] are the references.

Thank you.