Fundamentals of Protein Chemistry
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Module - 12
Special Topics in Protein Chemistry
Lecture - 56
Oxidative Stress in Proteins

In our final module of the course on fundamentals of protein chemistry, there are special topics in protein chemistry that are going to be discussed. We will start off with oxidative stress in proteins, this will be followed by a special lecture on enzymatic cleavage, followed by intrinsically disordered proteins and then viral proteins and in the last class we will give an overview of the topics that have been covered in the course.

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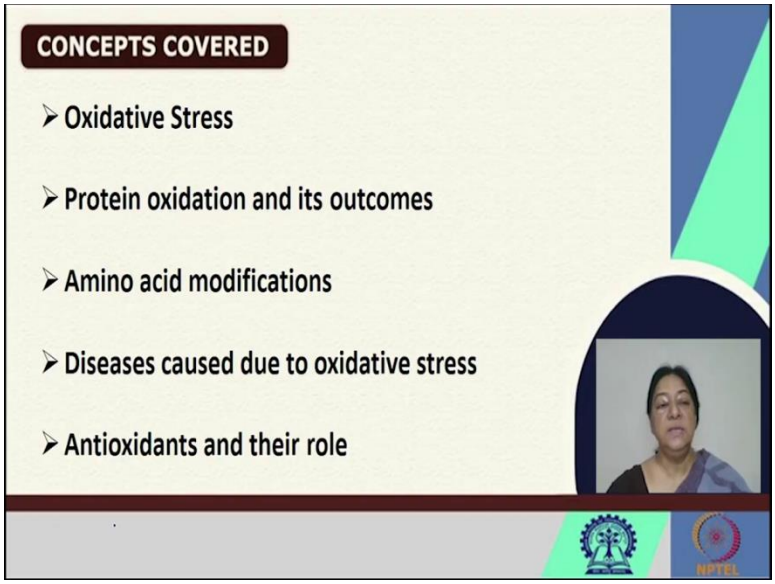

In this lecture we will look at the concepts covered in terms of oxidative stress, protein oxidation, what we mean by protein oxidation, specific amino acid modifications, the diseases that are

caused due to oxidative stress specific antioxidants and their role to combat oxidative stress and the damages that they cause.

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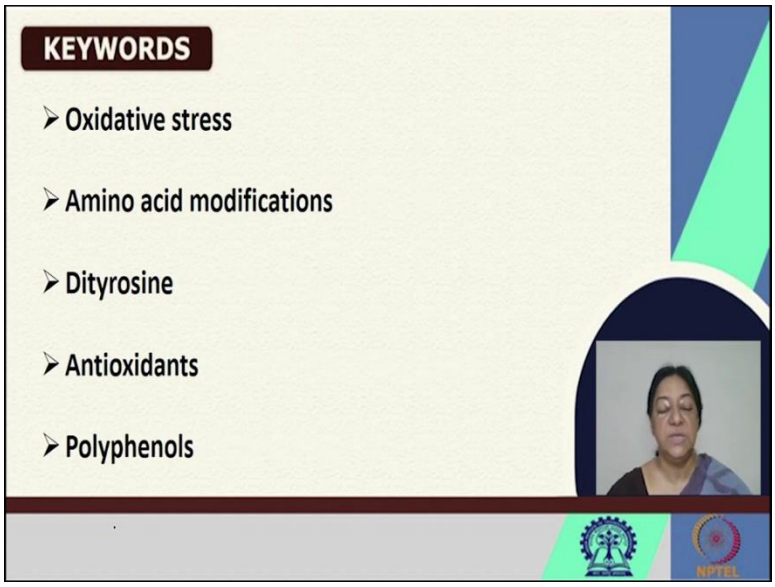

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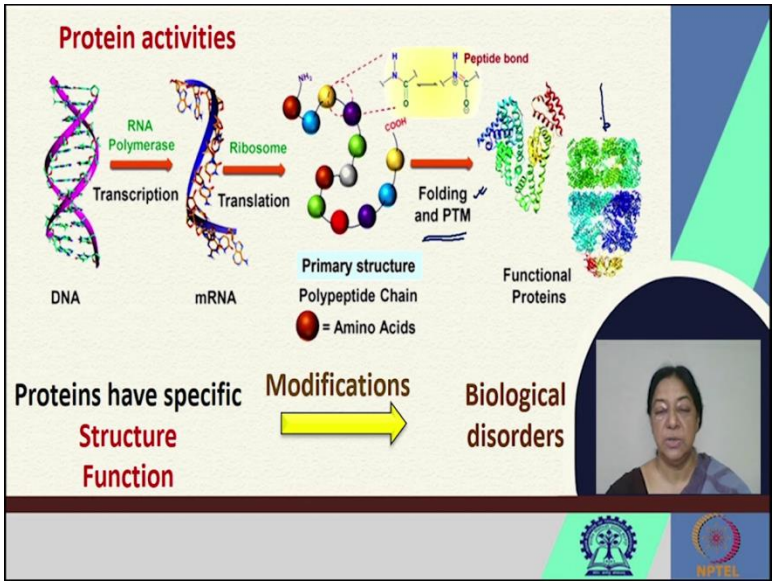

These [refer to slide] are specific keywords that we will be looking at as the lecture is delivered. When we look at proteins, we know the central dogma of biology from DNA to RNA to protein. Following this after folding, there is post translational modification and folding may lead to specific types of functional proteins; they could be enzymes, they could be structural proteins as we have seen during the course.

In addition to that there may be post translational modifications again, for specific functions that are required. For the chaperone proteins that we studied in a previous lecture, we know how that can assist in an improper folding process, to get the protein to fold properly.

So in our discussion here, when we try and understand the protein activities, we know that the proteins have a specific structure and associated with that, a specific function. Now modifications of the structure in any manner, can give biological disorders.

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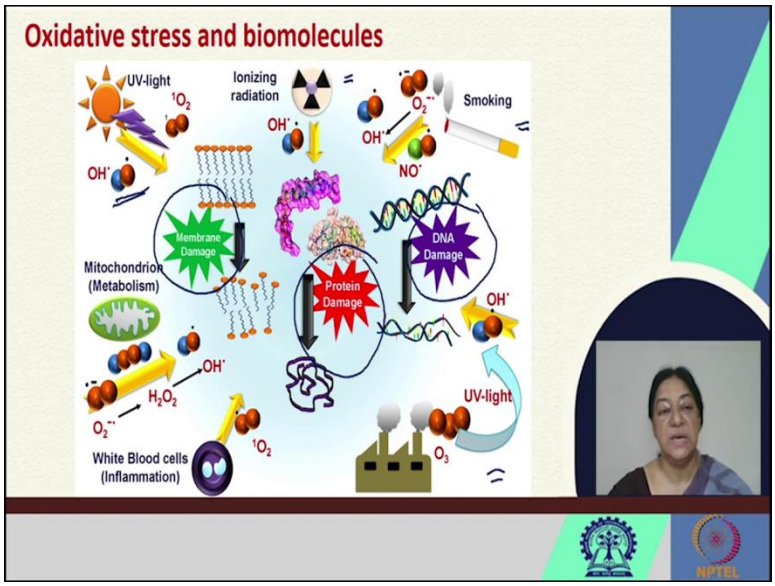

If we look at oxidative stress and biomolecules in general, there are several aspects that can affect biological macromolecules. So whether we are looking at variations in terms of UV light, ionizing radiation, smoking, UV light from other sources; any such sources can give rise to DNA damage, membrane damage and most importantly in this case, protein damage.

This protein damage can occur in a manner that is going to disrupt the structure and in turn disrupt the function of the protein. So it will not be able to perform the specific activity it has been designed to do.

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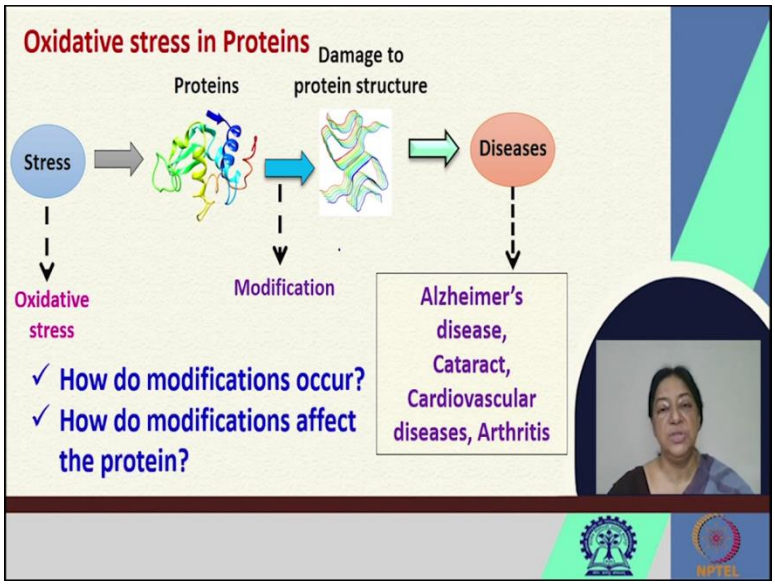

If we look at oxidative stress in proteins, we will understand what we mean by oxidative stress. There is damage to the protein structure, this oxidative stress leads to modification and this leads to disease; diseases such as Alzheimer's disease, cataract, cardiovascular diseases, arthritis and so on. The question is how do these modifications occur and how do the modifications affect the protein?

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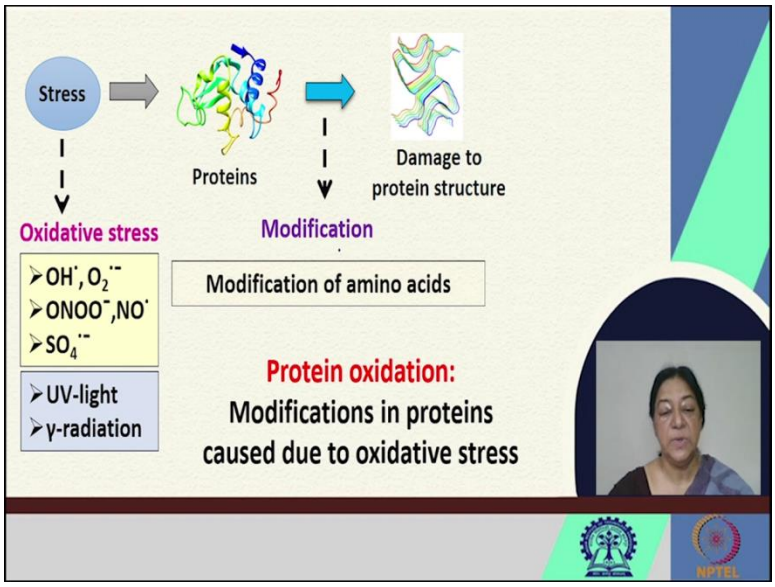

If we look at the stress and the proteins and the damage to the protein structure, the oxidative stress can be due to the presence of UV light gamma radiation and is usually caused by several radical formations, as has been shown here [refer to slide]. The modifications that can occur due to the specific amino acid variations, depend upon the type of amino acid the type of protein and its structural components.

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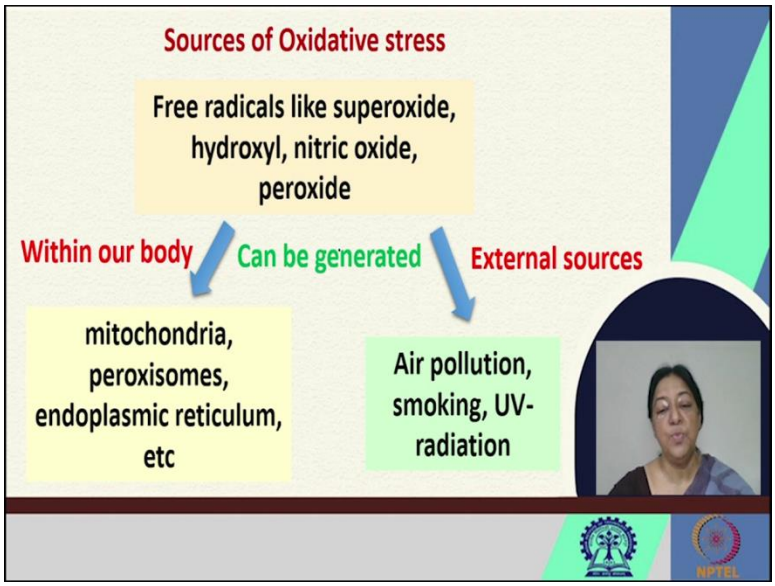

The modifications in proteins can occur at specific amino acids, which we will be looking at in a moment. The sources of oxidative stress are free radicals such as superoxide, hydroxyl, nitric oxide, peroxide and these can be generated within our body due to specific biochemical reactions that occur in mitochondria, peroxisomes, endoplasmic reticulum and can also be generated from external sources such as air pollution, smoking and UV radiation.

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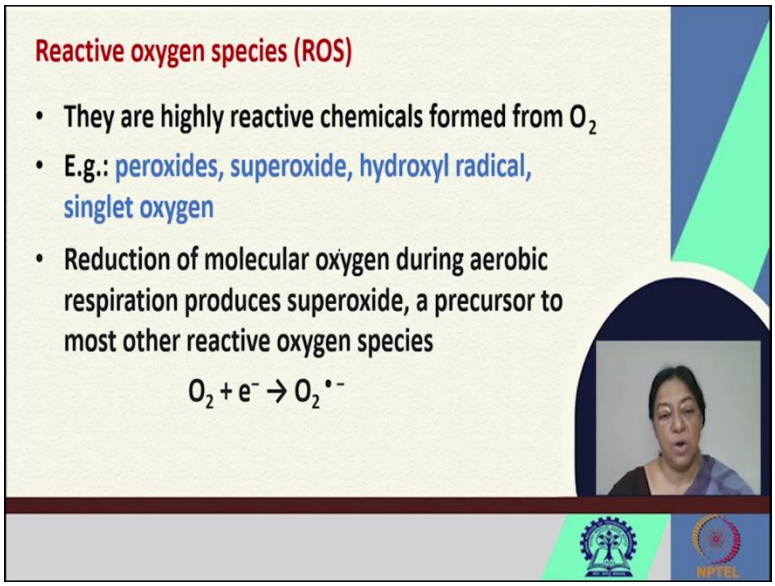

The reactive oxygen species that is formed, are highly reactive chemicals that are formed from oxygen. For example like was mentioned peroxide, superoxide, hydroxyl radical and singlet oxygen. The reduction of molecular oxygen during aerobic respiration, produces superoxide.

This superoxide is a precursor to most other reactive oxygen species and these are what cause damage.

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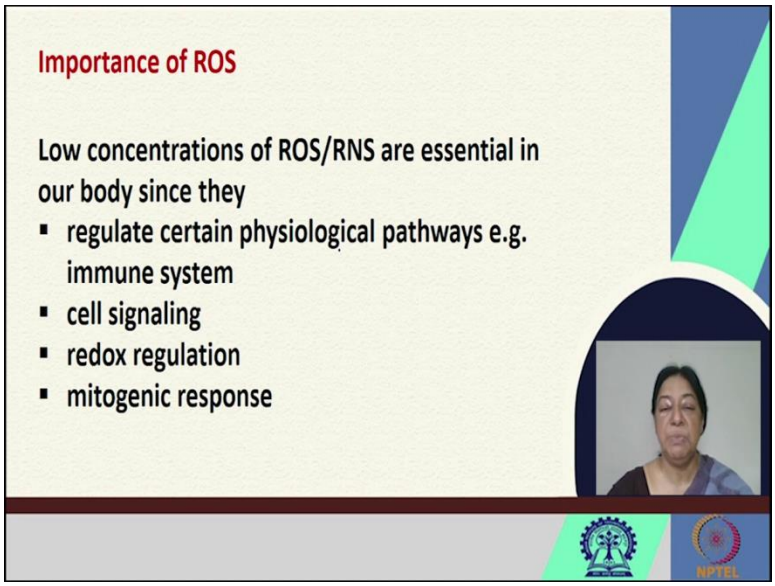

However, low concentrations of these ROS, the reactive oxygen species or the RNS, that is the reactive nitrogen species are essential in our body since they have certain functions in terms of a regulation of certain physiologic pathways for example, in the immune system, for cell signaling, for redox regulation and for mitogenic responses.

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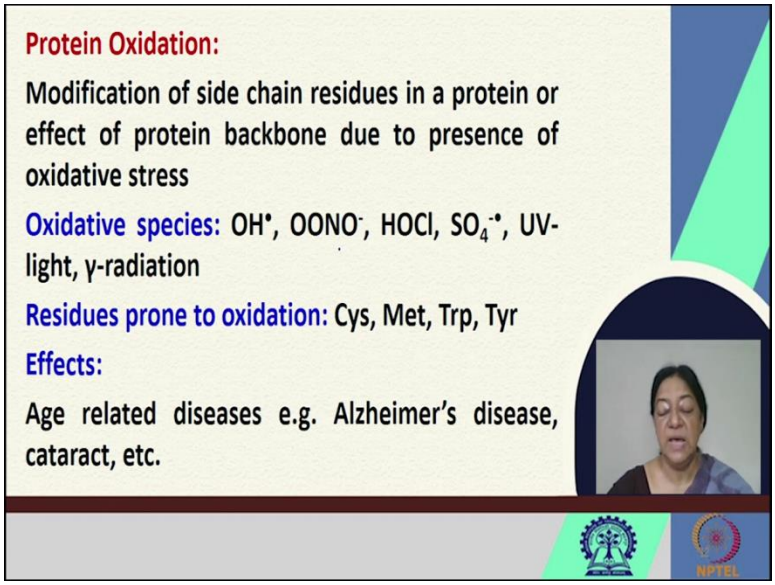

The protein oxidation therefore that occurs, is a modification of a side chain residue in a protein that can affect the protein backbone due to the presence of oxidative stress in the terms of the

components that we looked at, whether they are ROS or RNS. So the oxidative species are these and the residues prone to oxidation mostly are cysteine, methionine, tryptophan and tyrosine. The effects are age related diseases as was mentioned.

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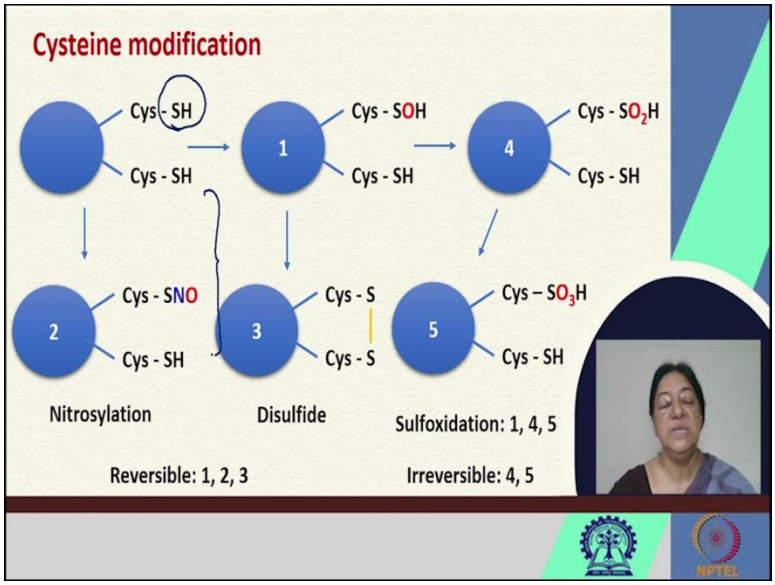

If we look at cysteine modification, we want to see what are the modifications that are possible in these residues. For cysteines we know that we have the sulfur containing side chain; this can form disulfide bonds. Now we realize the formation of the disulfide bond or the reduction to the cysteine, is a redox type of reaction where we have specific components possible, specific variations possible, due to this nitrosylation, due to oxidation, due to disulfide formation, due to other types of components.

In nitrosylation, sulfoxidation and disulfide formation some of these specific reactions are reversible in nature. However, some of them are irreversible resulting in permanent damage to the cysteine residues in terms of its functionality; in terms of its ability to form disulfide linkages.

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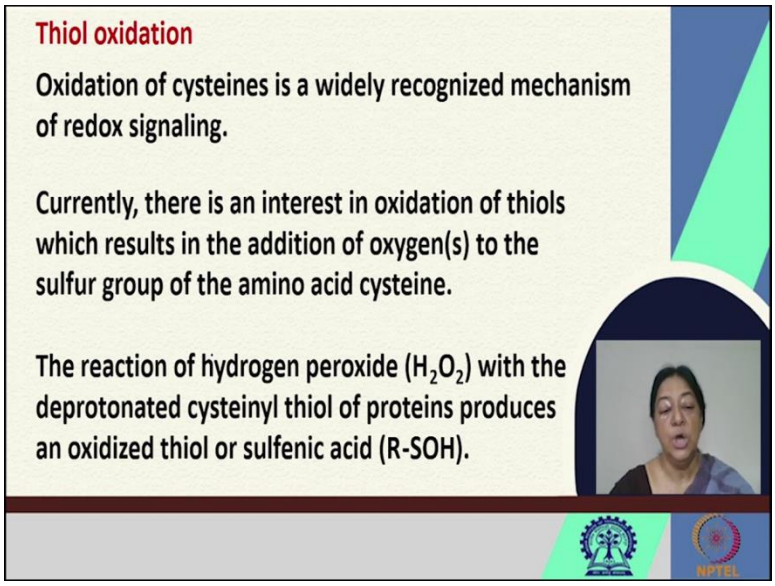

So thiol oxidation, that is an oxidation of the cysteines, is a widely recognized mechanism of redox signaling as is understood from the variations possible. There is interest of the thiols which results in the addition of oxygens to the sulfur group of the amino acid cysteine. The reaction for example of hydrogen peroxide with the deprotonated cysteinyl thiol of proteins, produces an oxidized thiol or sulfenic acid.

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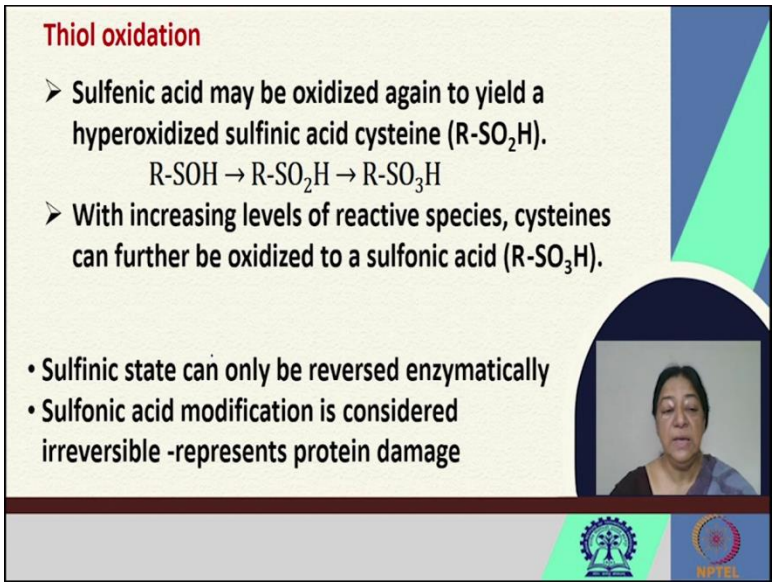

There are different possibilities that can occur in thiol oxidation. Sulfenic acid for example, may be oxidized again to yield a hyper oxidized sulfenic acid cysteine R-SO_2H. This in turn with increasing levels of reactive species, they can be further oxidized to what is called sulfonic acid R-SO_3H. So sulfinic state can be reversed enzymatically, but the sulfonic acid modification is considered irreversible and this results in protein damage.

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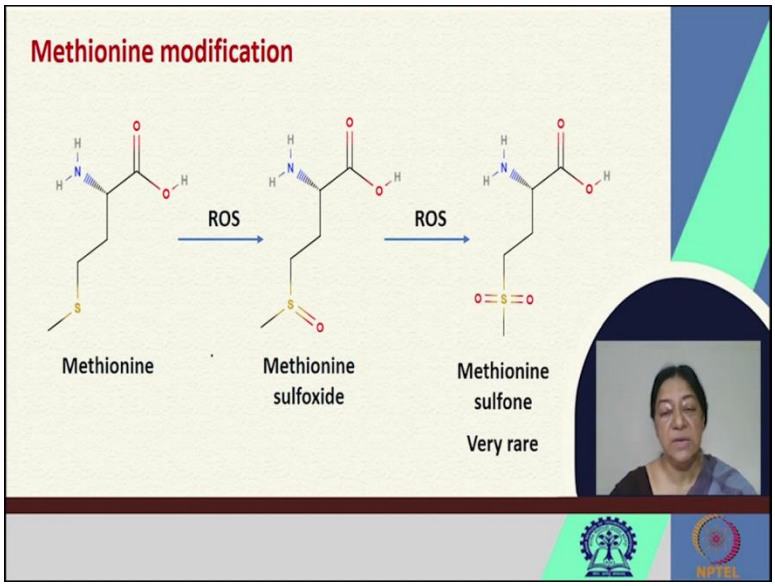

Methionine modification is also possible due to the presence of ROS, where we get methionine sulfoxide and further ROS can give me thionine sulfone, but the sulfoxide part is more common.

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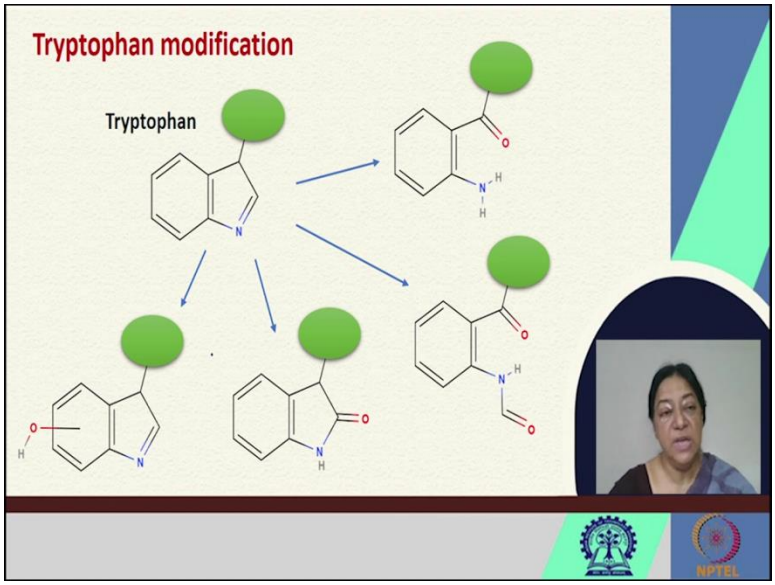

In tryptophan modification, we know that the tryptophan has an indole moiety as its side chain. Tryptophan modifications can occur due to the presence of the reactive oxygen species, giving variations in their structure and a disruption of the indole ring.

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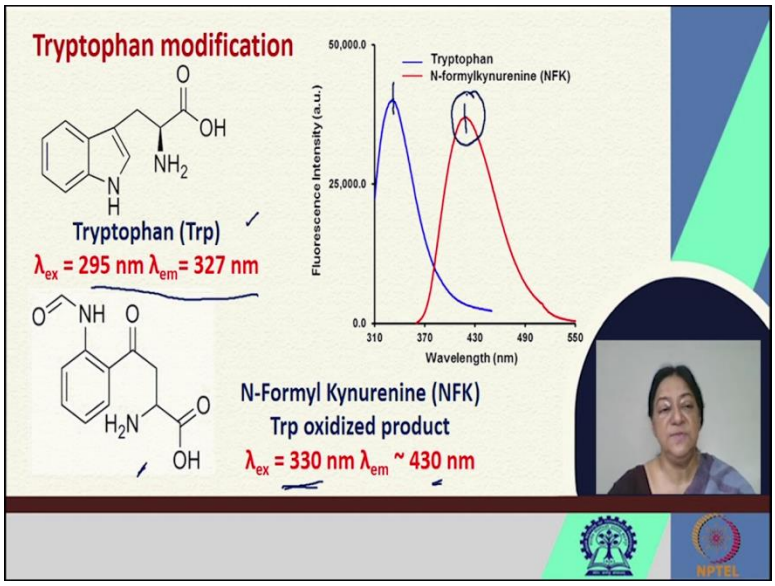

The tryptophan modifications can give a specific product known as N-Formyl Kynurenine, NFK. This is a tryptophan oxidized product which has the structure shown [refer to slide] and it has specific spectral characteristics for example, as in tryptophan we know the excitation can be a 295 nm with an emission around 330 nm, depending upon the conditions. In this case the excitation of NFK is around 330 nm and the emission is around 430 nm.

What this means is that if we have the presence of NFK in a species due to the tryptophan modification, it can be easily monitored by fluorescence spectroscopy. This gives us an indication or gives us an idea of what is called a biomarker, telling us that if NFK is present it means that the tryptophan has been modified and this can be tested for several modifications in proteins due to oxidative stress.

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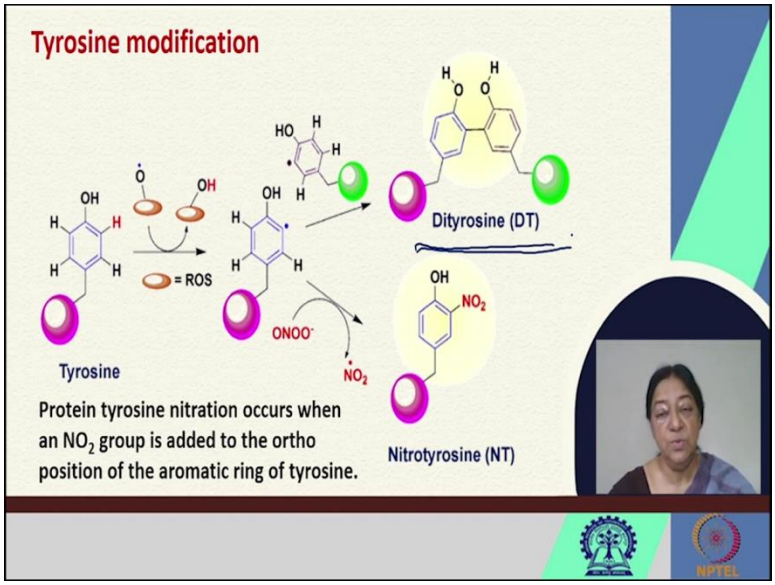

In case of tyrosine modification, there are specific possibilities that can occur. We can have dityrosine formation, where we have a cross linking of two side chains of tyrosine, two tyrosine radicals that can form dityrosine, which is another indicator in addition. There is also the formation of nitrotyrosine where the protein nitrous tyrosine nitration occurs when an NO_2 group is added to the ortho position of the aromatic ring of tyrosine, the side chain of tyrosine.

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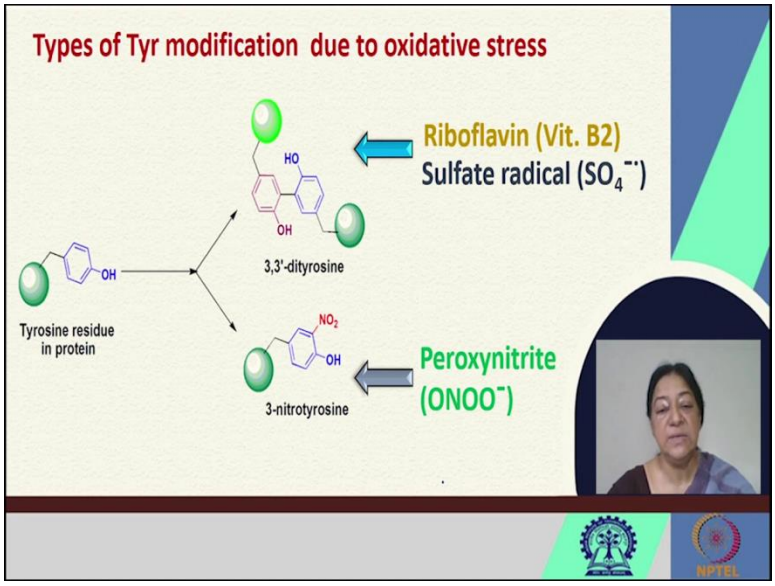

So the types of tyrosine modifications due to oxidative stress, can be in terms of 3, 3 prime dityrosine or 3 nitrotyrosine and these can be achieved in different ways as well.

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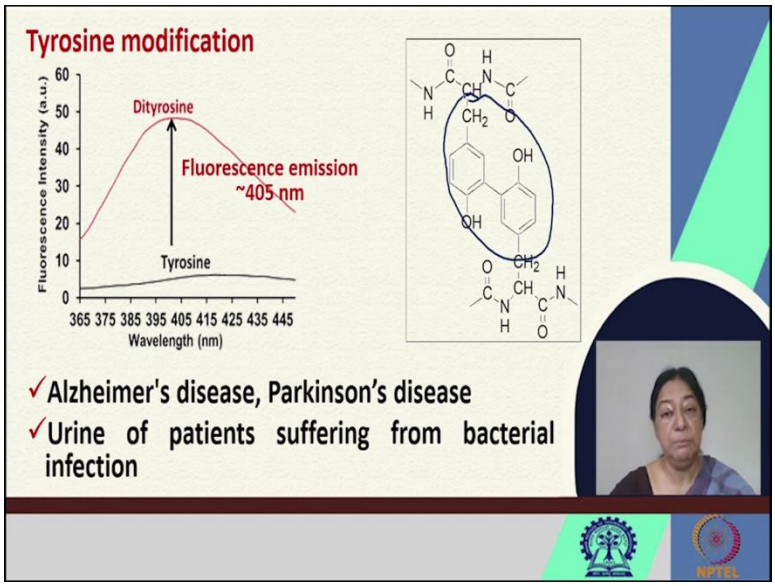

The dityrosine radical gives us a specific fluorescence signal due to the presence of the dityrosine formation and this again can be an indication of oxidative stress. The fluorescence emission is observed in Alzheimer's disease, parkinsons disease and also from the urine of patients suffering from any bacterial infection; so it can be an indicator.

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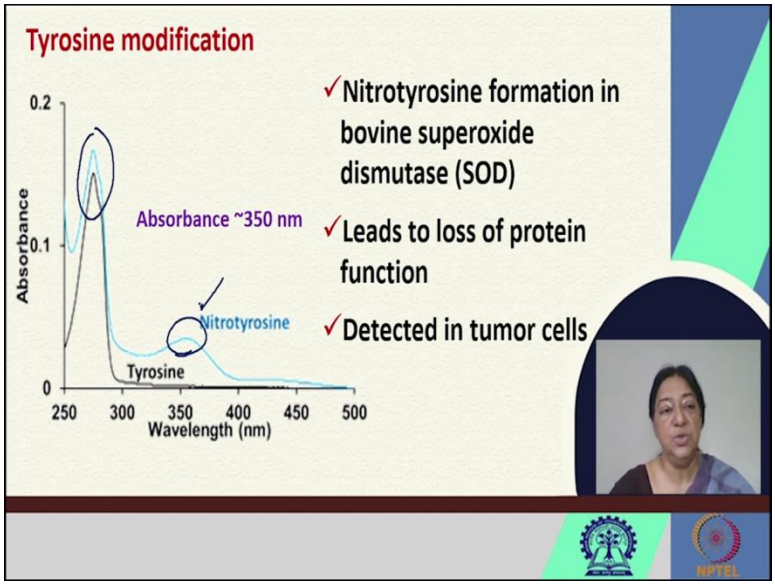

Similarly, we can have the absorbance signal of nitrotyrosine which is distinct from that of tyrosine. We have the signal here also, but this is an additional signal that is observed which can be used. So the nitrotyrosine formation in bovine superoxide dismutase has been seen to lead to loss of protein function and it can also be detected in tumor cells.

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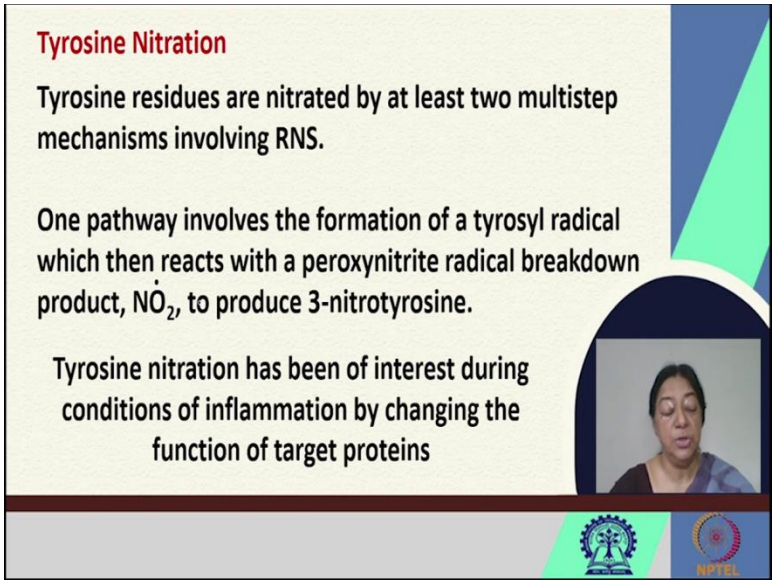

The tyrosine residues are therefore nitrated by at least two multistep mechanisms that involve reactive nitrogen species. One pathway involves the formation of a tyrosine radical, which then reacts with the peroxynitrite radical breakdown product, that is NO_2 dot, to produce 3-nitrotyrosine. This tyrosine nitration has been of interest during conditions of inflammation, by changing the function of target proteins.

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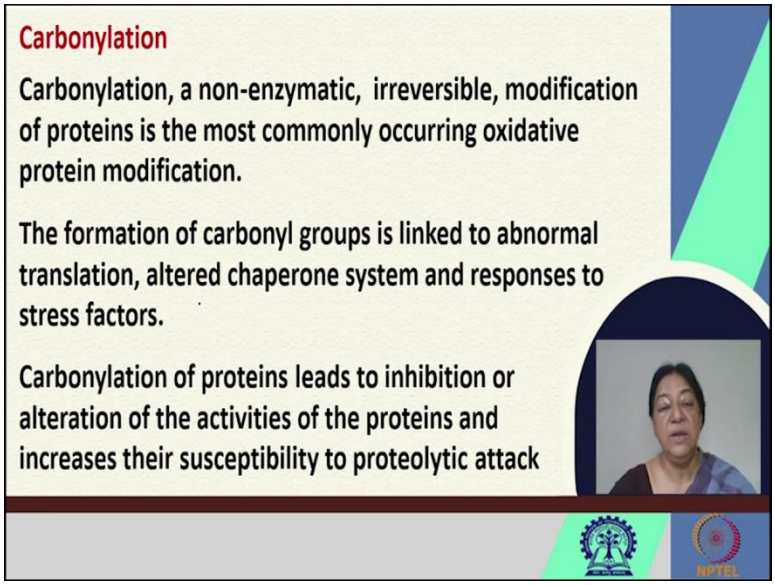

Carbonylation is a nonenzymatic, irreversible modification of proteins and is the most commonly occurring oxidative protein modification. The formation of the carbonyl groups is linked to abnormal translation, altered chaperone system and responses to stress factors. This carbonylation of proteins can lead to inhibition or alteration of the activities of the proteins and also increase their susceptibility to proteolytic attack.

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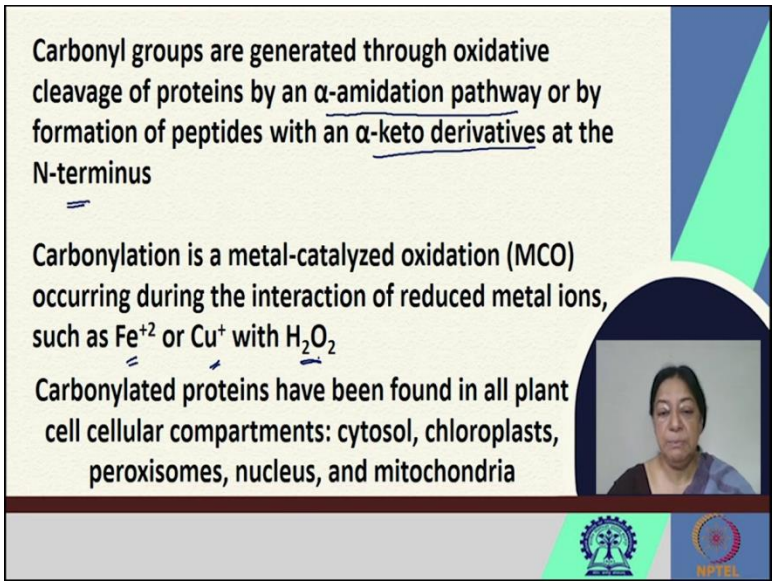

These carbonyl groups are generated through an oxidative cleavage of proteins, by what is called an α-amidation pathway or by the formation of peptides with an α-keto derivative at the N terminus.

So this is important and carbonylation is a metal catalyzed oxidation, occurring during the interactions of reduced metal ions for example, Fe^{+2} or Cu^{+} with H_2O_2. These carbonylated proteins have been found in all plant cell cellular compartments; for the example in cytosol, chloroplasts, peroxisomes, nucleus and also mitochondria.

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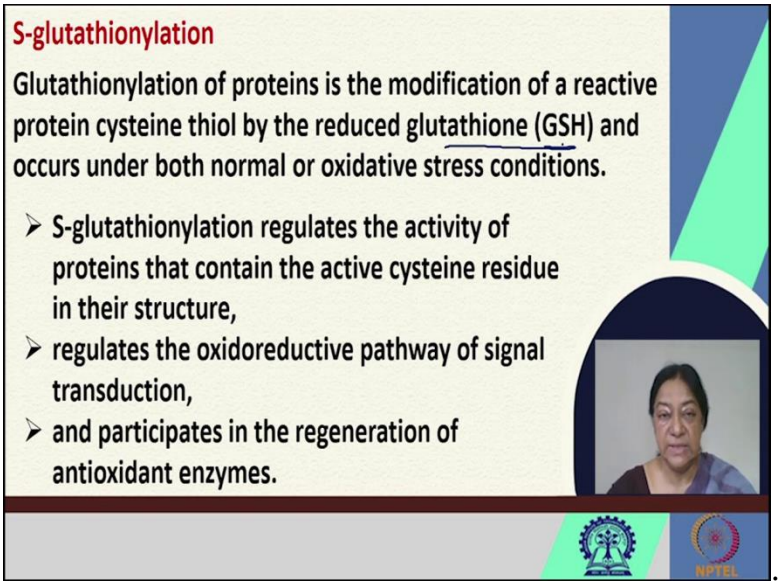

Another change is S-glutathionylation. This is the modification of a reactive protein cysteine thiol by reduced glutathione and occurs both in normal as well as oxidative stress conditions. This S-glutathionylation regulates the activity of proteins, that contain an active cysteine residue

in their structure. They regulate therefore the oxidoreductive pathway of signal transduction and they also participate in the regeneration of antioxidant enzymes.

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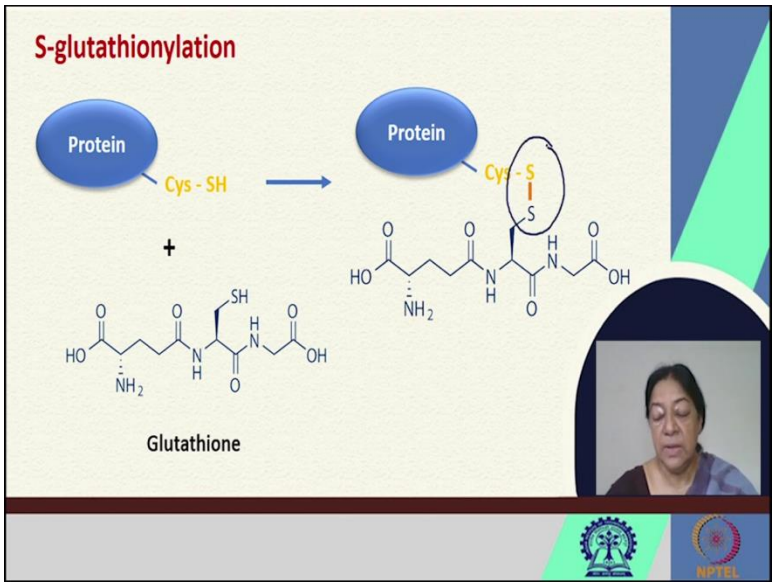

So if we have the protein that has this [refer to slide] particular cysteine residue with the free thiol, then this is glutathione that is now going to interact to form this disulfide bond. This then prevents the disulfide bond formation with other cysteine residues, in the formation of what we call a cystine.

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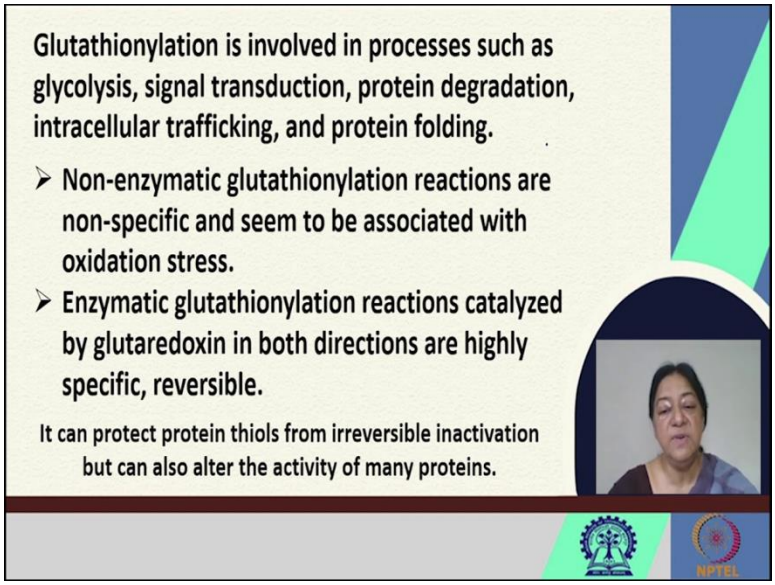

So this is involved in processes such as glycolysis, signal transduction, protein degradation, intracellular trafficking and also protein folding. The non-enzymatic glutathionylation reactions

are non specific and they are associated with oxidative stress. The enzymatic reactions are catalyzed by glutaredoxin in both directions, extremely specific and reversible as well. This can protect thiols from irreversible inactivation, but can also alter the activity of many proteins once the adduct is formed.

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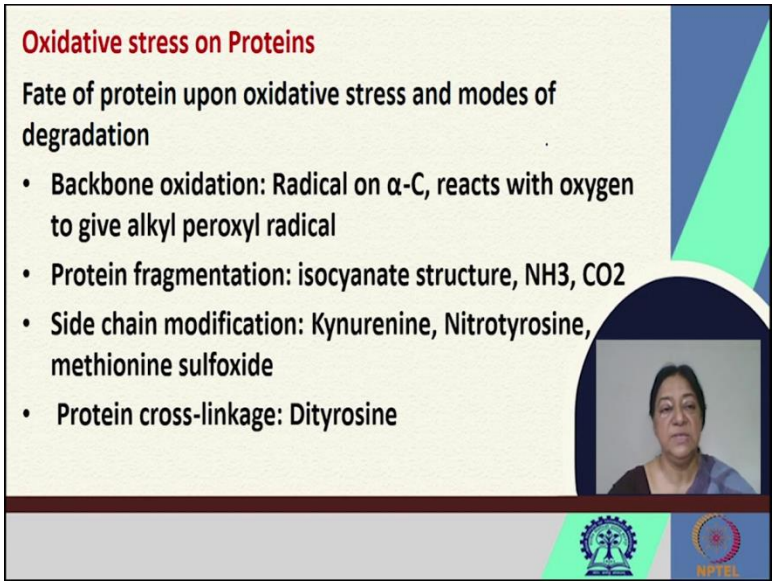

If we look at oxidative stress on proteins, there is a specific fate of the protein upon oxidative stress and there are modes of degradation. There may be backbone oxidation, where a radical could act on an α-C, react with oxygen to give an alkyl peroxide radical; protein fragmentation may occur; side chain modifications as we saw in terms of tryptophan, tyrosine, methionine and cysteine; and protein cross linkages in terms of the cross linking, based on two tyrosine radicals.

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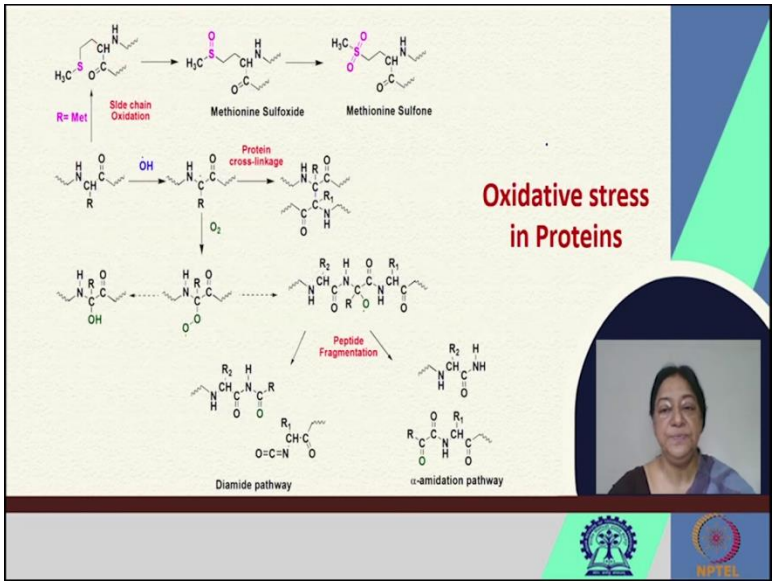

So oxidative stress in proteins can be summarized here [refer to slide], where we have an ammunition pathway, peptide fragmentation, protein cross linking and other side chain oxidative processes.

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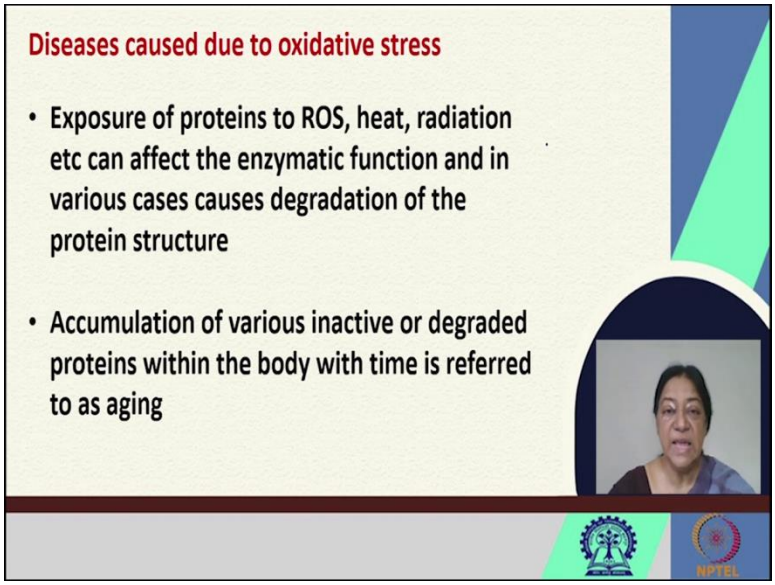

If we look at the diseases caused due to oxidative stress, there is an exposure of proteins to reactive oxygen species heat, radiation etcetera, that can affect the enzymatic function and in turn cause degradation of the protein structure. So the accumulation of various inactive or degraded proteins within the body with time, is referred to as an aging process.

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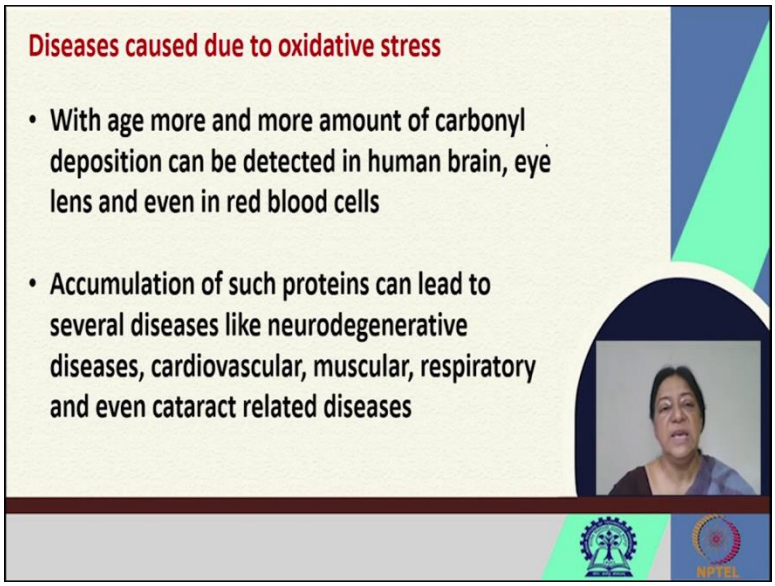

With age more and more amount of the carbonyl deposition can be detected in the brain, the eye lens and even in red blood cells. This accumulation of such modified proteins due to oxidative stress, can lead to various diseases.

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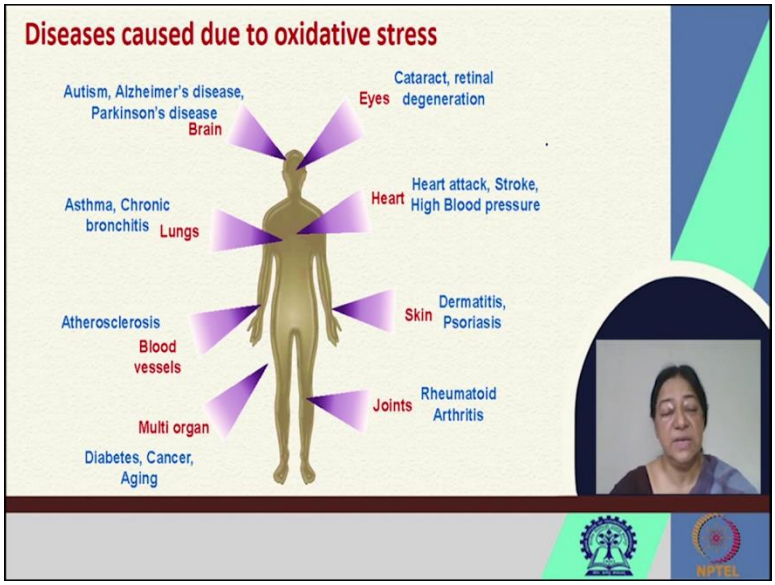

There are several diseases that are caused due to oxidative stress starting from cataract, Alzheimer's disease, asthma, specific heart attacks, stroke, atherosclerosis, diabetes, rheumatoid arthritis and so on.

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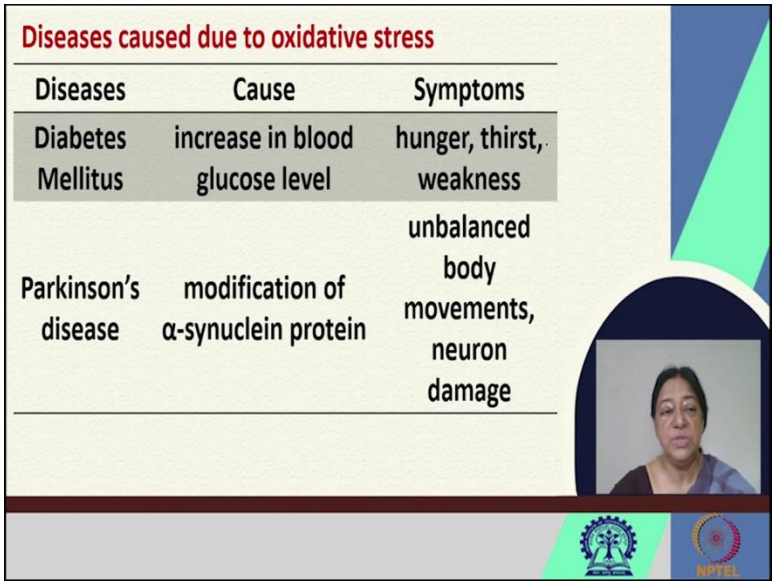

The specific proteins that are present and the causes due to the oxidative stress in proteins can lead to specific diseases and the specific symptoms are also given here [refer to slide].

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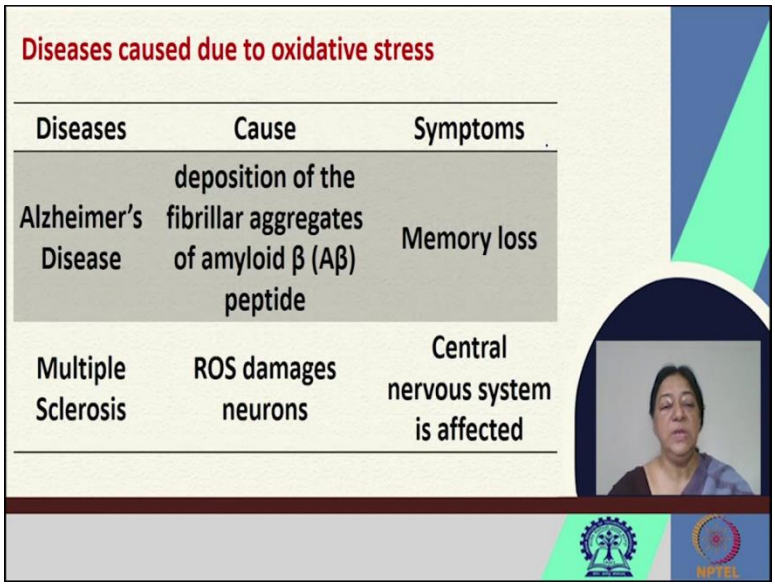

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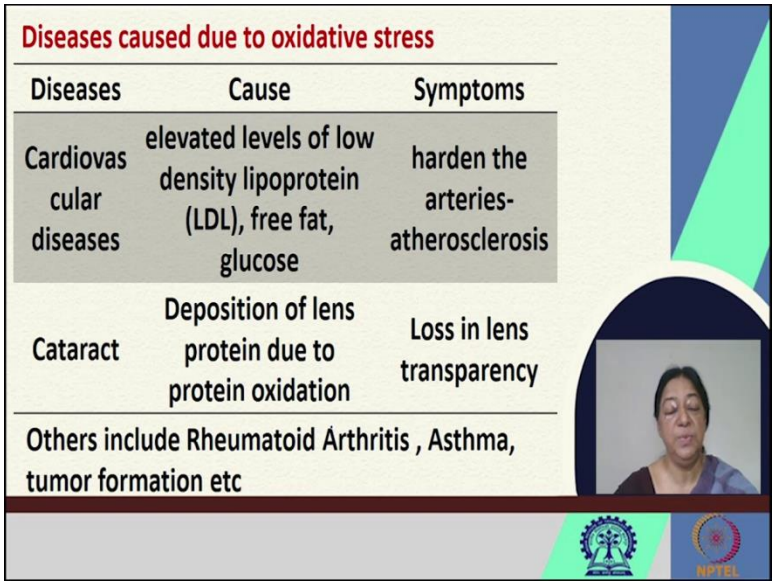

So we look at the deposition of specific amyloid peptide fragments, damaged neurons and also specific proteins that could be modified due to this oxidative stress process.

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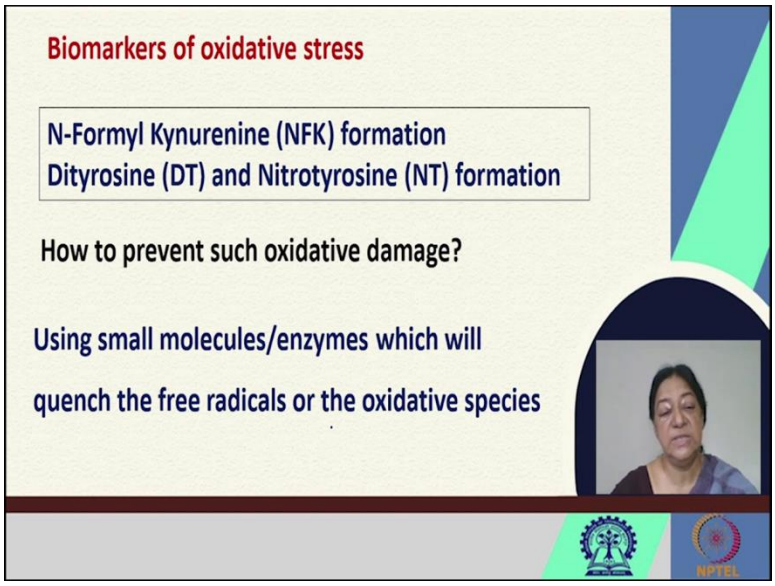

If we look at biomarkers of oxidative stress, we can look at NFK, dityrosine or nitrotyrosine formation. So the possibilities of modifications in proteins can occur through backbone modifications, can occur through specific amino acid modifications and these can lead to variations and disease.

The question is how to prevent such oxidative damage. This is possible by using small molecules of specific enzyme, that can quench these free radicals. But we also need to know that the presence of the free radicals is required for certain specific biochemical reactions.

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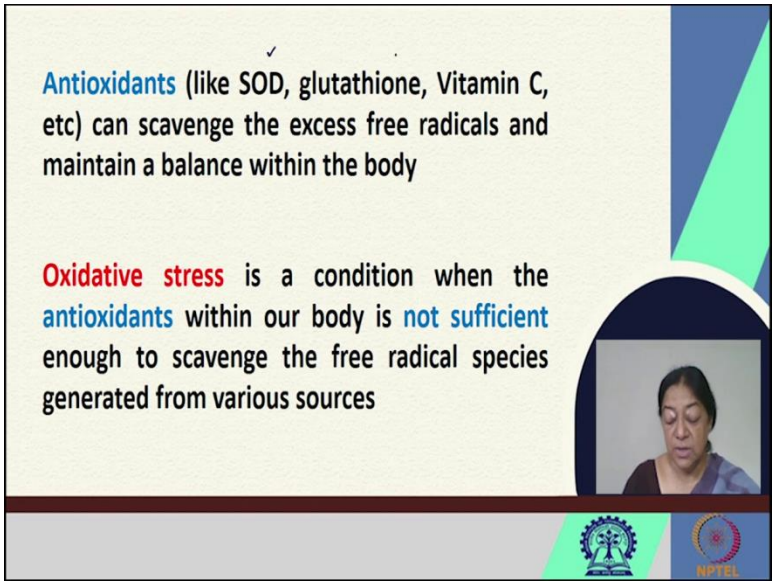

There are antioxidants like superoxide dismutase, glutathione, vitamin C etcetera, that can scavenge the excess free radicals and maintain a balance between the body. But oxidative stress

occurs when the antioxidants within the body are not sufficient enough to scavenge these free radicals that are generated by the various sources.

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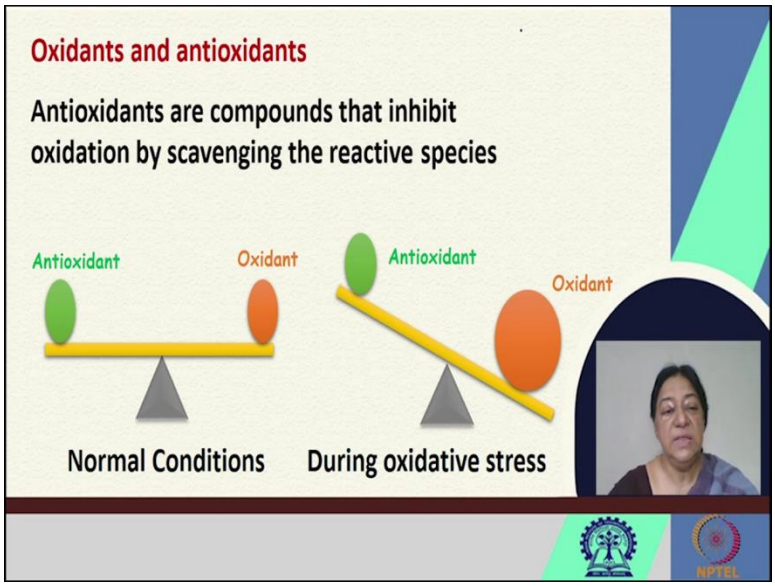

So effectively what happens, in normal conditions there would be a balance between the antioxidant and the oxidant. However during oxidative stress, we have a predominance of the oxygens and the antioxidants are compounds that would inhibit the oxidation, by being able to scavenge the reactive species.

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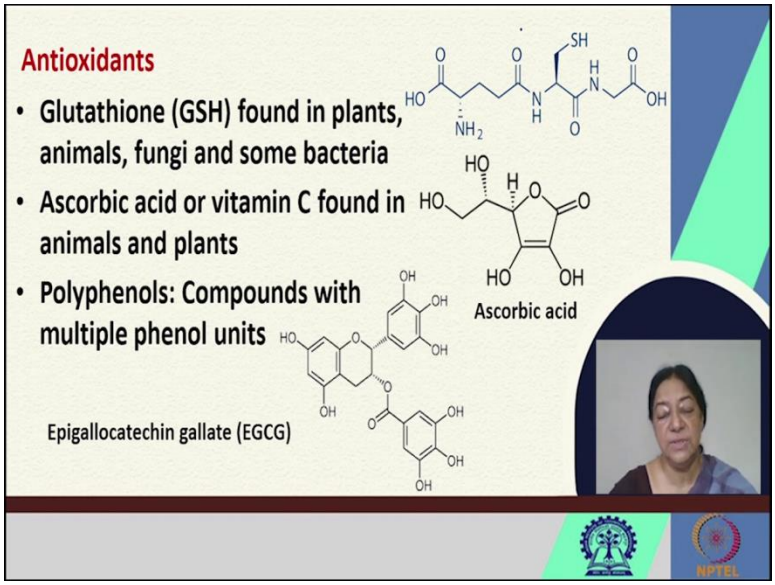

Glutathione as we just looked at, is found in plants, animal, fungi and some bacteria, is one such example. Ascorbic acid or vitamin C found in animals and plants and also polyphenols that are

compounds with multiple phenol units and some of these are a subject of a lot of research, where by looking at antioxidant activity on proteins we get an idea, either from dietary constituents or from enzymatic issues.

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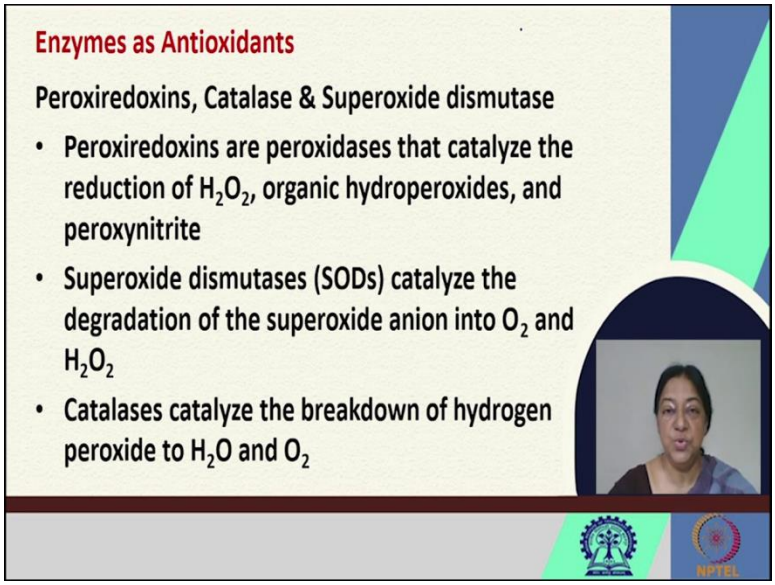

If we look at enzymes as antioxidants, there are peroxiredoxins, catalase and superoxide dismutase. Peroxiredoxins are peroxidases that catalyze the reduction of H_2O_2 organic hydroperoxides and peroxynitrite. Superoxide dismutases, these catalyze the degradation of the superoxide ion into oxygen and H_2O_2. The catalases catalyze the breakdown of hydrogen peroxide to H_2O and O_2.

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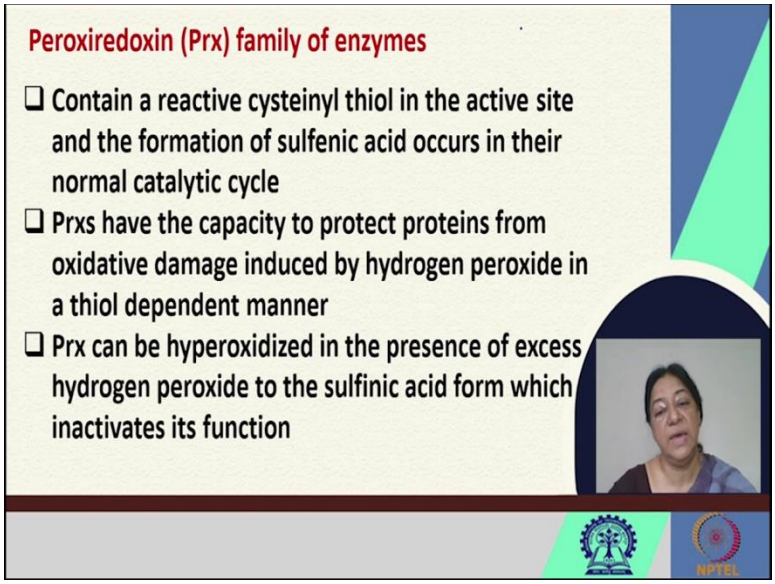

So if we look at the Prx family of enzymes, these contain a reactive cysteinyl thiol in the active site and the formation of sulfenic acid occurs in their normal catalytic cycle. These have the capacity to protect proteins from oxidative damage induced by hydrogen peroxide in a thiol dependent manner and they can be hyperoxidized in the presence of excess H_2O_2 to the sulfinic acid, which again inactivates its function.

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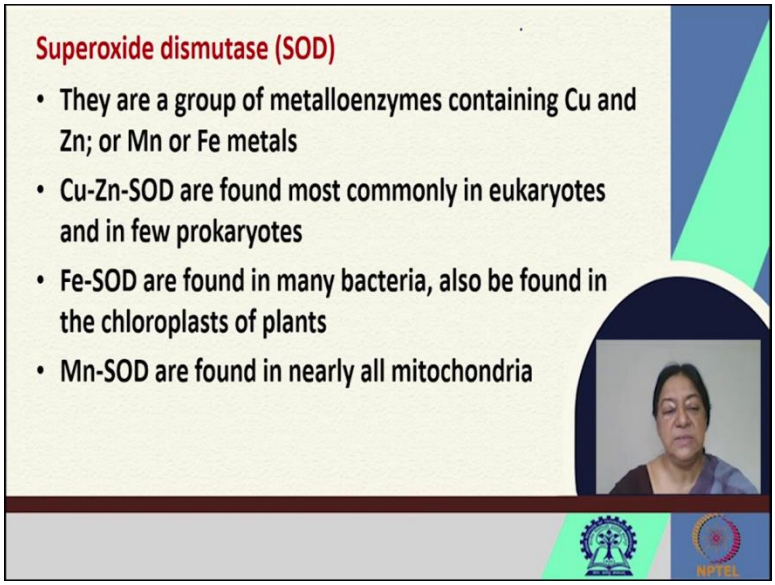

So there is a very delicate balance. If we look at superoxide dismutase, these are the group of metalloenzymes that contain copper, zinc or manganese and iron. Copper zinc superoxide dismutase are found most commonly in eukaryotes and in few prokaryotes and we have the variations in terms of the metals that are there in the superoxide dismutase. Some can be found in bacteria in plants and some in mitochondria.

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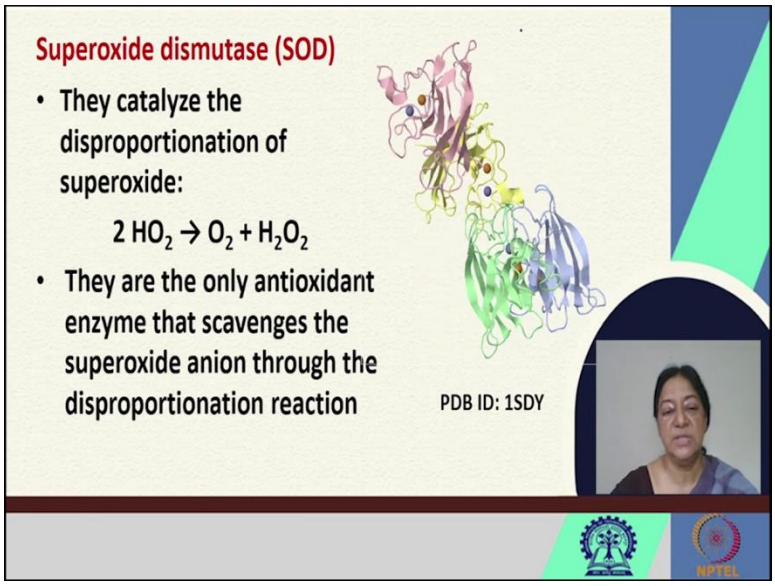

This [refer to slide] specific protein catalyzes the disproportionation of superoxide into O_2 and H_2O. They are the only antioxidant enzymes that are capable of scavenging the superoxide anion through this disproportionation reaction.

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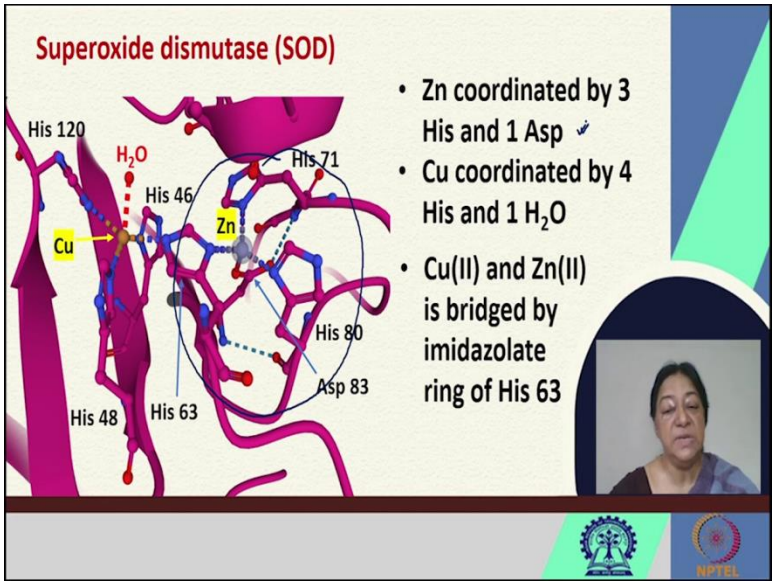

So, if we look at the specific active side as we saw in metalloproteins, we have the coordinations through the specific histidine residues and a specific activity due to this coordination. It is coordinated by three histidines and one aspartic acid, the copper is coordinated by 4 histidine and 1 H_2O. So, this is a copper zinc SOD superoxide dismutase and the Cu(II) and the Zn(II) are bridged by the imidazolate ring of His 63, as shown here [refer to slide].

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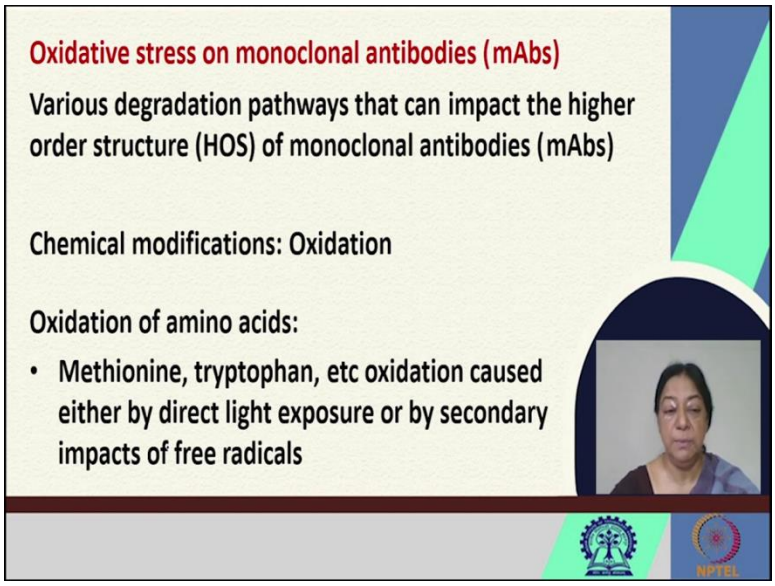

The oxidative stress on monoclonal antibodies is another important factor. There are various degradation pathways as we saw, that can impact the higher order structure of monoclonal antibodies. We had seen the structure of antibodies previously and how they are important in antibody antigen interactions.

So, chemical modifications in terms of oxidation may be possible and we looked at the oxidation of specific amino acids. Here for example methionine, tryptophan etcetera oxidation that can be caused by direct light exposure or by secondary impacts of free radicals.

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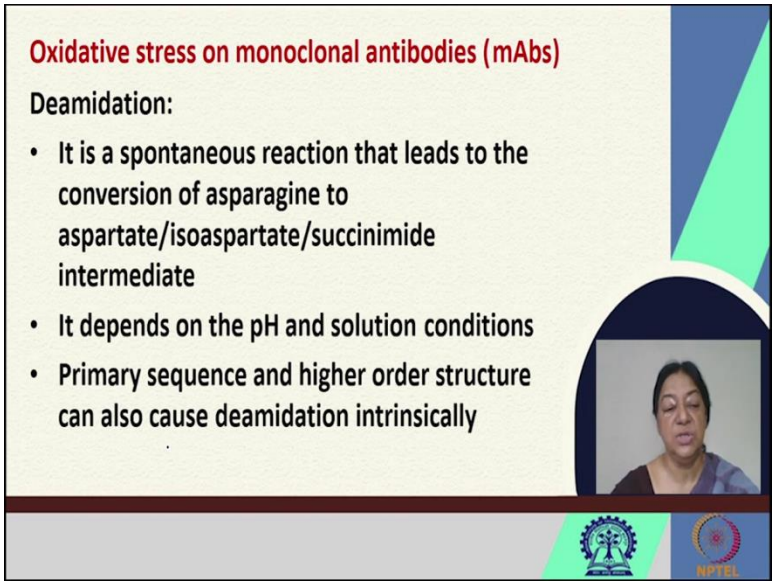

In case of deamidation, this is a spontaneous reaction that leads to the conversion of asparagine to aspartate, isoaspartate, succinimide intermediates. These depend upon the pH as well as the

solution conditions and the primary sequence and higher order structures can also cause intrinsic deamidation, which is an important factor in oxidative stress of these antibodies.

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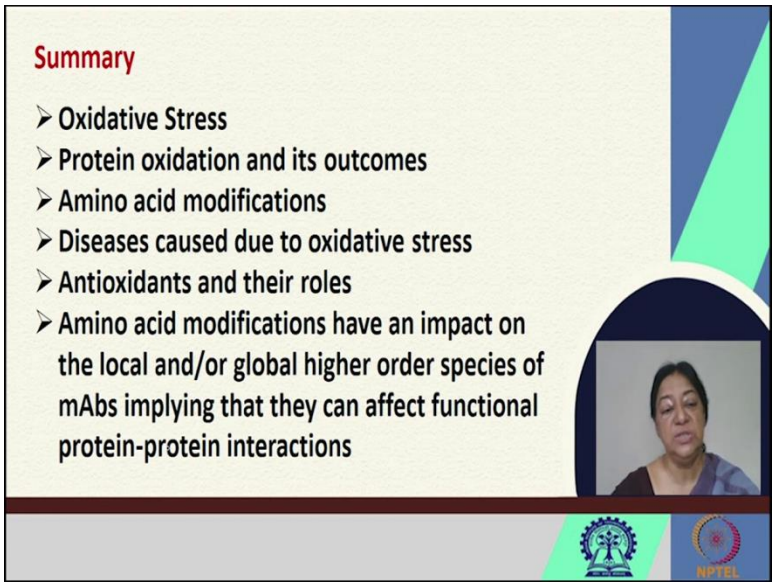

In summary what we looked at, we looked at oxidative stress in proteins, we understood that there were specific possibilities for the damage due to oxidative stress in terms of backbone modification, specific amino acid modifications that could also be used at biomarkers to understand the extent of oxidative stress.

The protein oxidation and its specific outcomes and how we have specific diseases due to these amino acid modifications and the role of antioxidants in combating any oxidative stress that may occur. The amino acid modifications we learnt have an impact on the local and/or global higher order species of the monoclonal antibodies and this is important because this means that they can affect functional protein-protein interactions.

In this specific topic or the specific topics that we will be considering for this module, are more research oriented to give us an idea of the open problems in protein chemistry. Not all of them are possible to be discussed here, but nevertheless to get some idea, the specific references and specific books available will be given.

Thank you.