Introduction to Biomolecules
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Lecture-26
Nitrogen Metabolism

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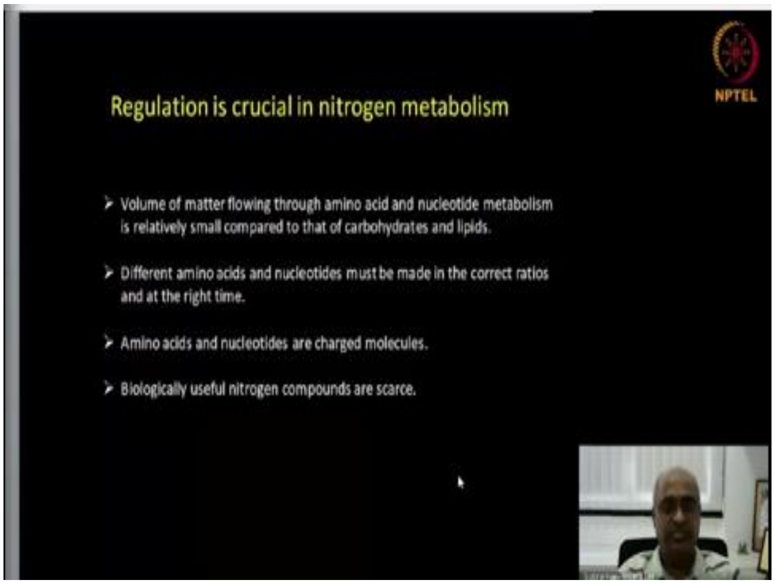

Regulation is crucial in nitrogen metabolism

- Volume of matter flowing through amino acid and nucleotide metabolism is relatively small compared to that of carbohydrates and lipids.
- Different amino acids and nucleotides must be made in the correct ratios and at the right time.
- Amino acids and nucleotides are charged molecules.
- Biologically useful nitrogen compounds are scarce.

Very good, so let us get started. So, in the last class we began on nitrogen metabolism and then I was sort of highlighting why nitrogen metabolism is tightly regulated? So, the reasons are shown in bullet forms in this slide and this slide is what we discussed last in the last class. And before that I told you the leguminous plants, that is the pulses, lentils and oil seeds, they play a major role in getting the atmospheric nitrogen into biologically usable form that is ammonia into the biological systems. So, today we will see how that process happens; nitrogen to ammonium, so that is the main thing we are going to look at today.

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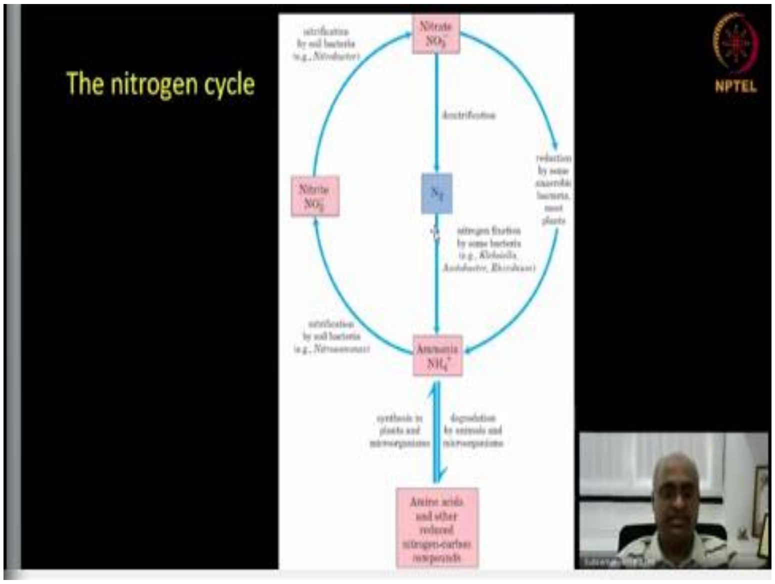

The nitrogen cycle

So, this slide sort of familiar already, we have looked at it earlier, so just for a quick refreshing. So, bulk of the nitrogen exist as free nitrogen molecule in the atmosphere, these 2 nitrogen atoms triple bonded, highly stable and enough to gas, bulk of the air is made up of nitrogen. But this nitrogen is not directly usable, for example this nitrogen does not become the amino group in our amino acids or the nitrogen incorporated into purine and pyrimidine rings or the porphyrin rings that we saw in the cytochromes.

So, for that the nitrogen needs to be reduced and that is done by bacteria, nitrogen fixing bacteria or like Klebsiella, Azotobacter, Rhizobium. So, these live in the nodule like structures in the roots of the legume plants and some of them are free soil bacteria as well. So, they reduce the nitrogen into ammonia, this ammonia can be incorporated into amino acids by the plants and microorganisms.

And when they die and degrade they return ammonia back into the soil. But this free ammonia does not remain as ammonia in the soil, primarily because these nitrifying bacteria in the soil Nitrosomonas. So, these are extremely abundant, so therefore there is no free ammonia in the soil, nowhere you get the ammonia smell in the soil except in uncleaned bathrooms, where the urea of urine gets converted into ammonia by bacterial actions.

So, these nitrifying bacteria convert the ammonia into nitrate, first nitrite and then to nitrate**.** And so this nitrate is what is abundantly in the soil which the plants can use, so reduction by anaerobic bacteria and most plants to ammonia and that ammonia is incorporate into amino acids**.** So, this is the nitrogen cycle and some of the nitrates are converted and free nitrogen is returned to the atmosphere by the action of denitrifying bacteria.

So, that is the reason you need to continuously convert nitrogen into the reduced form. Because these denitrifying bacteria take the nitrate out of the soil and return into the atmosphere. So, you have in this cycling of nitrogen**,** so we saw the cycling of carbon, hydrogen, oxygen through photosynthesis and oxidative phosphorylation in bulk of the lectures that we had so far. So, here we are looking at the cycling of the nitrogen**.** The primary points are nitrogen gas is reduced.

(Professor Student Conversation Begins: 04:42)

Can you scale up the size? The size ok, just 1 second.

(Professor Student Conversation Begins: 04:55)

So, you have the nitrogen here which is reduced to ammonia by nitrogen fixing bacteria**.** So, most of the ones that are relevant to us are the nitrogen fixing bacteria living in the root nodules of leguminous plants.

So, that is where we are going to spend most of our time today. And this ammonia is converted into nitrate by the nitrifying soil bacteria**.** And therefore free ammonia does not exist in the soil and this nitrate is the one that is reduced that ammonia and used by the plants. Plants meaning, they fix incorporated into amino acids**,** for example transamination of alpha-ketoglutarate which is an intermediate of TCA cycle will produce glutamate**.**

So, that is how nitrogen gets into the biological molecules. And some of the nitrates are converted back into nitrogen gas by the denitrifying bacteria. So, this is the cycling of nitrogen and this ammonia incorporated into amino acids by plants and microorganisms is the one from which we get our nitrogen. So, we do not use ammonia directly, only plants and microorganisms do that and for us the relevant ones are the plants.

And from the plants you get all the amino acids that you need, that is how we get the nitrogen. So, we do not take nitrogen in any of these forms, we do not take directly nitrogen gas, we do not use nitrate, we do not use ammonia also, we only use in the form of amino acids given to us by the plants. So, any of you think, who is thinking that you get your protein from meat, you should think again, so animals get the protein from plants. So, if this nitrogen cycle is clear to you, we will move on, our focus is this nitrogen to ammonia.

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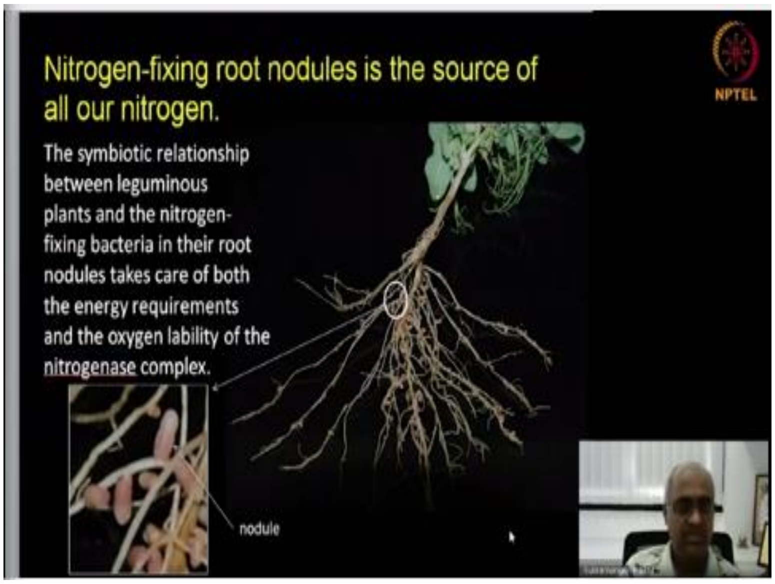

Nitrogen-fixing root nodules is the source of all our nitrogen.

The symbiotic relationship between leguminous plants and the nitrogen-fixing bacteria in their root nodules takes care of both the energy requirements and the oxygen lability of the nitrogenase complex.

So, this is a pea plant, the field pea, it is related to Mendel's garden pea. So, this is the green parts of this plant which in Hindi they call mutter which becomes available now, like November, December season in plenty that is this plant. So, this has the in the pod you have multiple seeds and those seeds are what we use as one of the leguminous seeds. And this is how other ones due to the chickpea, pigeon pea and so on like black gram, bengal gram, green gram, all the grams the lentils that we eat in all those plants you have this.

This particular image I took from a field in Kanpur where they grow mutter which is the field pea. If you look at the root system and the circled area up close here on the root you have these oval shaped structures. So, these are the nodules and you can see there are lots of them in the plant. And these nodules are root plant structures these themselves are not bacteria, these are the plant structures.

And inside them you have the nitrogen fixing bacteria Rhizobium species live there. And they exist in as shown here symbiotic relationship with the leguminous plants and here the symbiosis, the gain for the plant is fixed nitrogen that is in the form of ammonia for use. And the benefit for the bacteria is the root provides all the energy requirements for reducing nitrogen. And also the nitrogen reducing enzyme, nitrogenase is very sensitive to oxygen and the oxygen is removed away from this bacterial nitrogenase and protected by the plant produced components.

So, therefore plants provide both energy requirements and they take care of the oxygen lability of the nitrogenous complex. So, that is how bacteria benefits from the plants, plant benefits by getting the fixed nitrogen.

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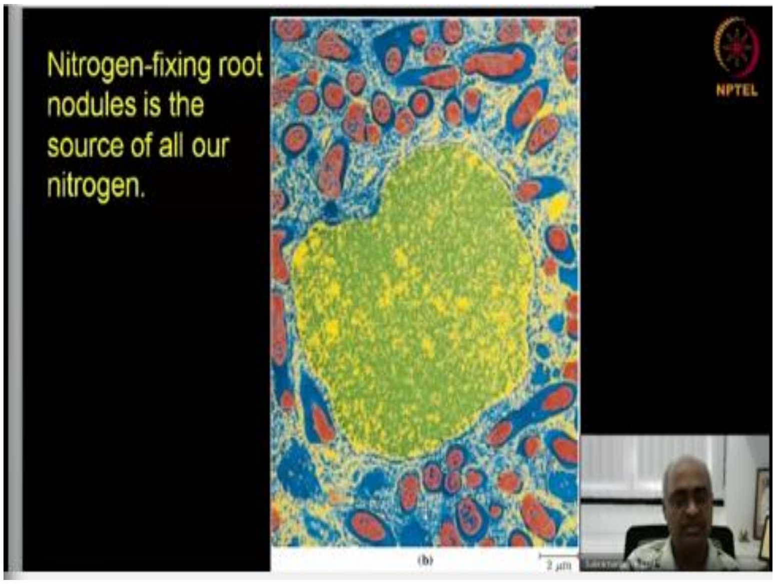

Nitrogen-fixing root nodules is the source of all our nitrogen.

So, if you take a cross section of this and look at one single cell, so this is a single cell of the nodule structure. So, this is the nodule structure if you cut this and use a light microscope and look at a very close structure like 2 micron is the scale bar here. So, this is the large nucleus of that cell and in it is cytoplasm you have a lot of red structures, these are those Rhizobium bacteria.

So, they are surrounded by a bacterial protective outer cover and so that is the blue coloured structure. So, these are the bacteria, these are the nitrogen fixing bacteria. So, these are living in the cytoplasm of the plant cell, so this whole thing is inside one single cell, so you have only one nucleus and the cytoplasm you have so many bacteria. So, these are the bacteria that actually provide you the nitrogen that is there all over your body in form of amino acids, porphyrin range, purine range, pyrimidine rings and pyridoxal phosphate, so one of the vitamins as well.

So, many of them have nitrogen in the amine, chlorine, niacin, riboflavin, thiamine all of them. So, all those nitrogen come from the activity of these red coloured bacteria here. So, this is staining, the bacteria does not have colour.

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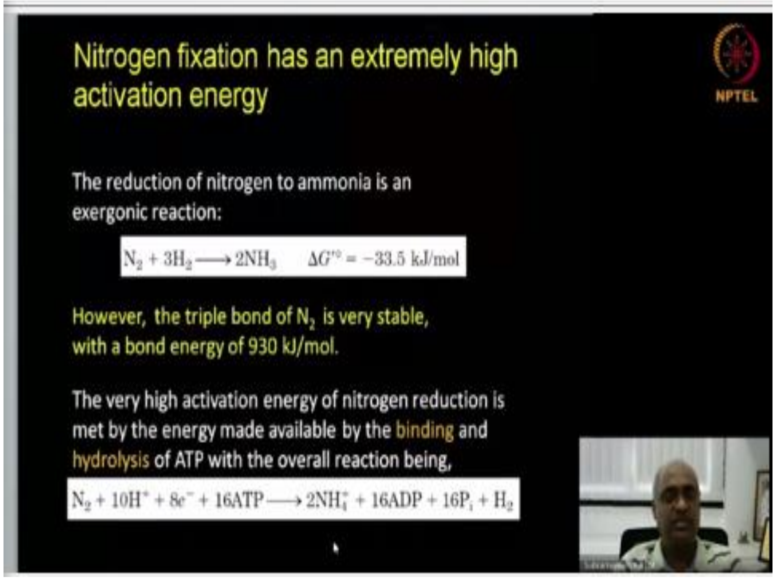

Nitrogen fixation has an extremely high activation energy

The reduction of nitrogen to ammonia is an exergonic reaction:

$$N_2 + 3H_2 \longrightarrow 2NH_3 \qquad \Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

However, the triple bond of N_2 is very stable, with a bond energy of 930 kJ/mol.

The very high activation energy of nitrogen reduction is met by the energy made available by the binding and hydrolysis of ATP with the overall reaction being,

$$N_2 + 10H^+ + 8e^- + 16ATP \longrightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$$

So, what do they do, what the bacteria does? It has an enzyme that can do this reaction, summarized here**,** nitrogen reduced to ammonia. So, as you can see here this is actually with a large negative delta G. However, this does not spontaneously happen due to extremely high activation energy that is because the triple bond is very stable and to break that you need this kind of an energy 930 kilojoules per mole is the bond energy of the triple bond between the 2 nitrogen atoms in the nitrogen molecule.

And due to that it does not readily happen and the enzyme nitrogenase which we are going to see in a minute manages to overcome this high activation energy barrier by binding of ATP**.** When the ATP binds the active site, the various non-covalent interactions that happen between the active site amino acid side chains and the ATP molecule provides lot of binding energy there**.** So, that reduces the reduction potential of the enzyme, so that it can readily donate an electron**.**

So, electrons flow from molecules with low reducing potential to molecules with high reducing potential**,** so electron affinity difference. That is one way by which ATP binding provides contributes to overcoming this activation energy barrier. And second of course which is familiar to us ATP hydrolysis itself precedes with the negative delta G and that energy is also available. So, here the ATP provides energy by 2 important ways and just to stress that point I have coloured them separately**.**

So, the summary is this, nitrogen + 10 protons, 8 electrons you get and then you have 16 ATP hydrolysis to provide 2 molecules of ammonia**.** And just to take care of the stoichiometry of this catalysis, 2 protons are reduced into a hydrogen molecule, so that happens as part of it. So, essentially you need 6 electrons for the reduction of the nitrogen molecule into 2 ammonia molecules. So, this is the overall reaction catalyzed by this enzyme.

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Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

Contains two enzymes:

1. Dinitrogenase reductase
2. Dinitrogenase

So, here is the enzyme itself in it is full glory. So, here you can count 1, 2, 3, 4 different structures you see here. So, this is actually one separate enzyme, this is called dinitrogenase reductase enzyme, it has 2 subunits they are identical, so this is homodimer enzyme. And then dinitrogenase itself, that is this one, so this is a hetero tetramer, so you have 2 grey subunits and 2 this light pink subunits.

So, this is the hetero tetramer, 4 polypeptide chains. And this is one more of the same this dinitrogenase reductase. So, 2-dinitrogenase reductase can associate with a single dinitrogenase complex, so totally you have got an 2 here times 2, so 4 and this itself 4. So, you have about 8 subunits polypeptide chains making this whole complex. And it has the non polypeptide moieties popping up in this and that alone is shown here, so that you have here.

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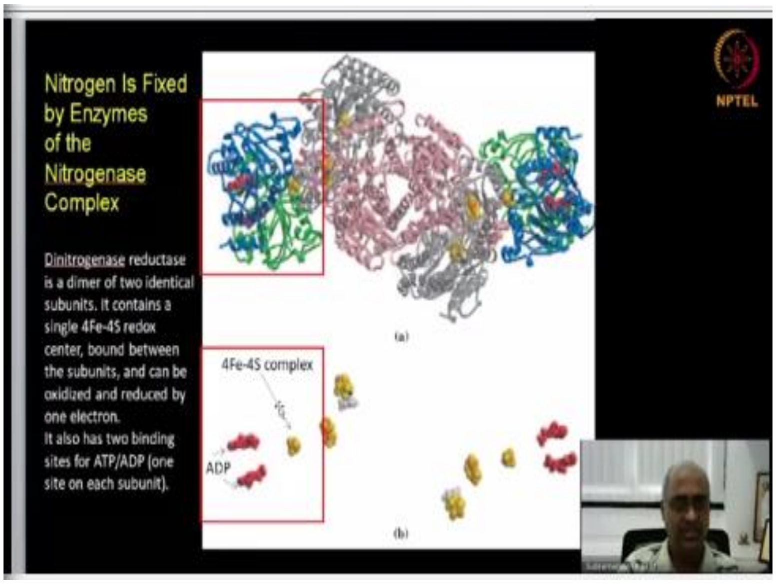

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

Dinitrogenase reductase is a dimer of two identical subunits. It contains a single 4Fe-4S redox center, bound between the subunits, and can be oxidized and reduced by one electron.
It also has two binding sites for ATP/ADP (one site on each subunit).

So, first let us focus on the dinitrogenase reductase itself, so it has a single 4 ions and 4 sulfur complex. And it also has the site for binding 2 ATP or ADP depending on whether it is already hydrolyzed or ATP is just binding. So, 2 molecules of ATP or ADP and 4 Fe-4S iron sulfur complex, so this is what is present in this. So, this is the one that is going to accept electrons from an electron donor and this is going to transfer the electrons to the dinitrogenase itself, which is going to reduce the nitrogen into ammonia.

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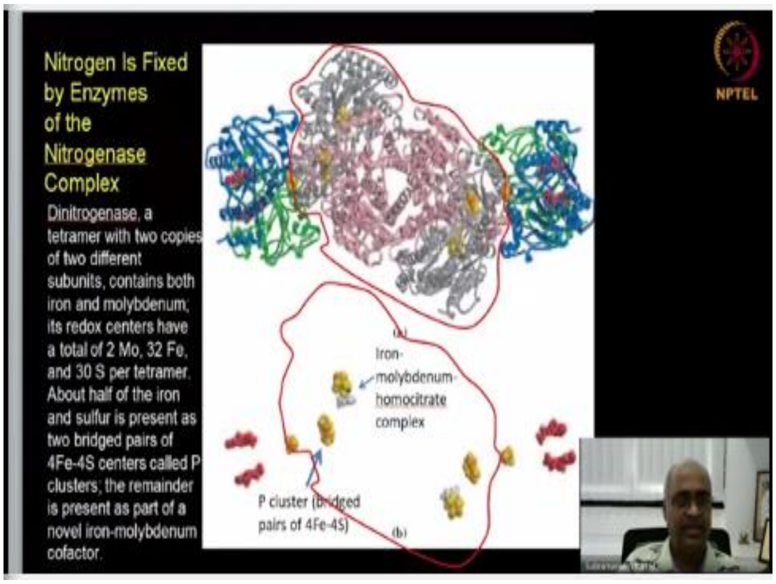

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

Dinitrogenase, a tetramer with two copies of two different subunits, contains both iron and molybdenum; its redox centers have a total of 2 Mo, 32 Fe, and 30 S per tetramer. About half of the iron and sulfur is present as two bridged pairs of 4Fe-4S centers called P clusters; the remainder is present as part of a novel iron-molybdenum cofactor.

So, now let us look at the dinitrogenase complex, this I told it as a tetramer, it has 2 copies of 2 different subunits. And now we encounter a new metal iron, molybdenum. So, we have seen manganese and we have seen magnesium, we have seen iron, so now we have seeing molybdenum, I do not think we are going to encounter any more new metal iron in our biochemistry course, although cobalt plays a role in vitamin B12. But we are not going to discuss vitamin B12, the rest all the metals you have already encountered, so molybdenum is the latest you are seeing here.

So, this iron molybdenum complex shown here which is attached to homocitrate it is a modified citric acid. So, that complex is crucial for the activity of this enzyme. And in addition you have this 4Fe-4S complex which is called P cluster. So, it contains 2 of them here, so one here and one here, similarly this one also 2 complexes because it is basically identical, so this is the structure of the dinitrogenase.

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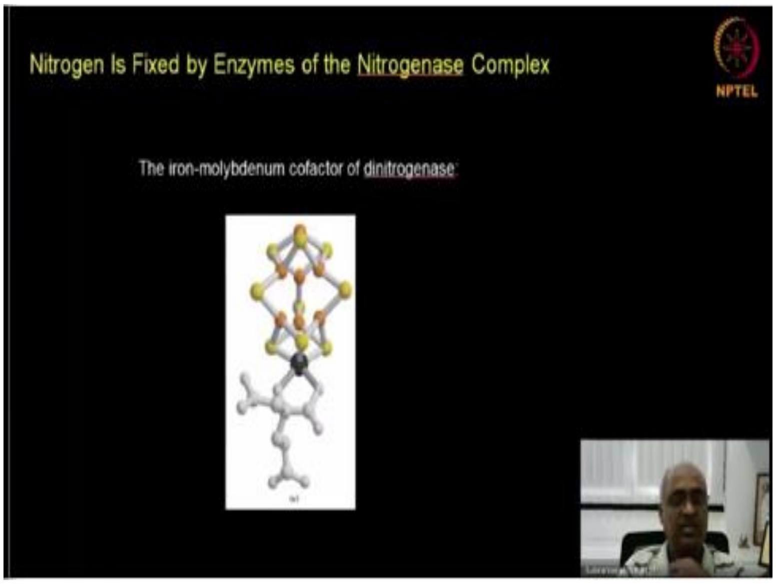

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

The iron-molybdenum cofactor of dinitrogenase

So, this is a close-up of this complex iron molybdenum homocitrate complex. Because this we are encountering only here and nowhere else. So, this shows the structure, this black one is the molybdenum**.** So, I actually we do not know why molybdenum is specifically chosen for this, like we do not have such an elaborate answer as we had it for manganese in the water splitting complex. So, here the orange ones are the irons and the yellow ones are the sulfur and this is the molybdenum and this is the homocitric this grey ones are the homocitrate.

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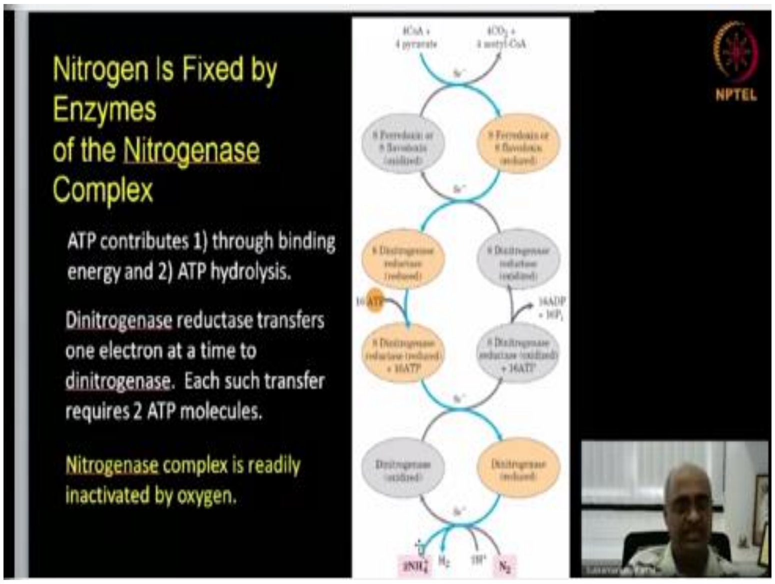

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

ATP contributes 1) through binding energy and 2) ATP hydrolysis.

Dinitrogenase reductase transfers one electron at a time to dinitrogenase. Each such transfer requires 2 ATP molecules.

Nitrogenase complex is readily inactivated by oxygen.

So, this shows the pathway of electrons. Say essentially from a donor**,** if the donor’s vary in this particular example from a particular bacteria, where it is pyruvate. So, pyruvate is the donor of the electrons, the electrons are first transferred to the ferredoxin. So, from the oxidized ferredoxin this dinitrogenase reductase takes up the electrons and becomes reduced. The electrons are taken up in the 4Fe-4S complex that we saw is part of this dinitrogenase reductase complex.

And then it binds the ATP molecules and you transfer the electrons to the dinitrogenase and the ATP is hydrolyzed and then it goes back to the oxidized form then it can do the cycle again. So, it takes 1 electron at a time and transfers 1 electron at a time to the dinitrogenase complex. So, every time what it does is, this one when it takes up an electron from the ferredoxin or reduced ferredoxin and oxidizing the ferredoxin in therefore.

And hydrolyzes the ATP, it transfers 1 electron to these centers, P cluster, iron molybdenum center. And once it transfers then this dissociates from this complex and goes away, so the oxidized version. Then it again goes through the cycle again and it takes up electrons. So, like that it makes 8 trips to provide the 8 electrons or if you have 2 of them then it goes 4 rounds. And for each electron transfer it requires 2 ATP, so 16 ATPs are hydrolyzed to provide the 8, out of the 8, 6 go to reducing the nitrogen and 2 of them combined with 2 protons to make the hydrogen**.**

So, this is how from a original electron donor electrons are transferred to the dinitrogenase complex by this dinitrogenase reductase enzyme. And then this one then reduces the nitrogen into ammonia. So, this I have already highlighted, ATP contributes through both binding energy and ATP hydrolysis. And dinitrogenase reductase, this again we have already covered, the other important specific thing about dinitrogenase is it is highly labile to oxygen**.**

So, oxygen readily gets the electrons and you do not reduce nitrogen and the nitrogen is complex gets inactivated. So, it is very sensitive to oxygen and oxygen inactivates this enzyme. So, this enzyme needs to be protected from oxygen for it is activity.

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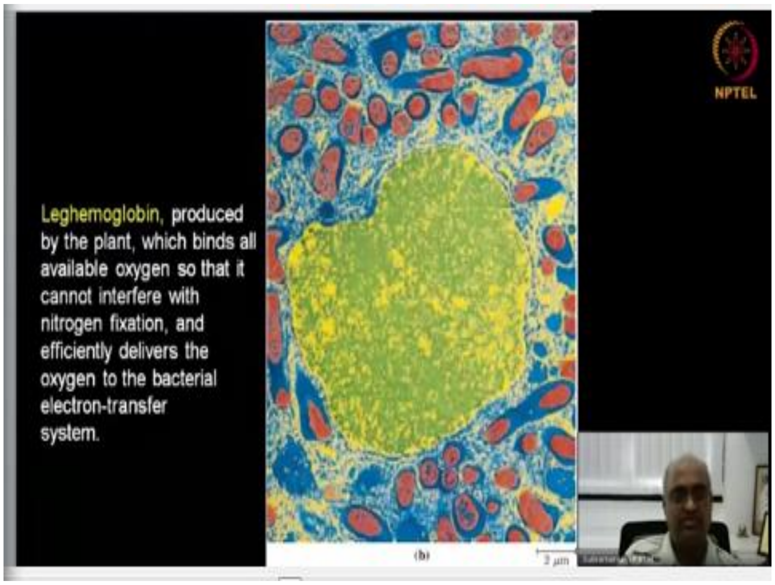

Leghemoglobin, produced by the plant, which binds all available oxygen so that it cannot interfere with nitrogen fixation, and efficiently delivers the oxygen to the bacterial electron-transfer system.

And that is achieved by leghemoglobin**,** so this is a hemoglobin kind of molecule except that instead of iron it contains magnesium. And this one present in the cytoplasm which backs this bacteria, takes up all the oxygen in the cytoplasm with this tightly binds oxygen. And by doing this not only it protects the nitrogenase from oxygen, it also efficiently delivers the oxygen to the bacterial electron transfer system, remember oxidative phosphorylation to produce energy.

So, therefore oxygen is made available for that electron transfer reaction and there it becomes what? So, leghemoglobin's main job is to protect the nitrogenous complex from the toxicity of oxygen**.**

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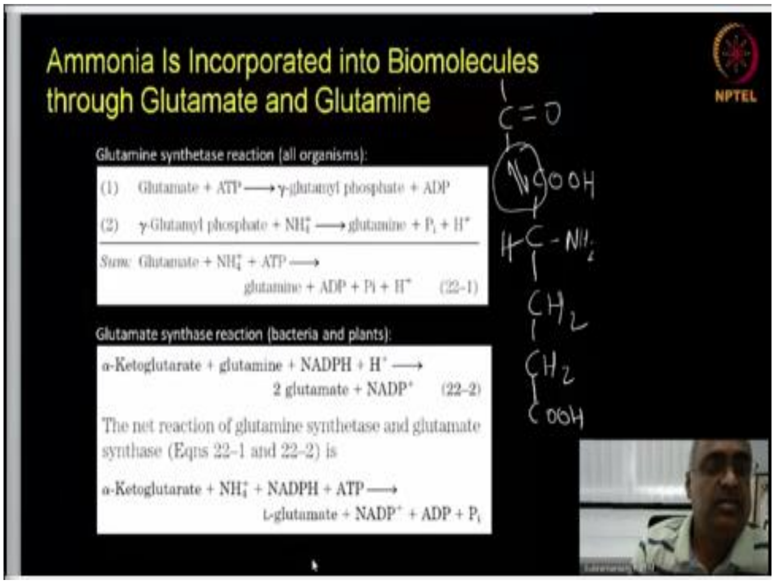

Ammonia Is Incorporated into Biomolecules through Glutamate and Glutamine

Glutamine synthetase reaction (all organisms):

(1) Glutamate + ATP $\longrightarrow$ γ-glutamyl phosphate + ADP

(2) γ-Glutamyl phosphate + NH_4^+ $\longrightarrow$ glutamine + P_i + H^+

Sum: Glutamate + NH_4^+ + ATP $\longrightarrow$ glutamine + ADP + Pi + H^+ (22-1)

Glutamate synthase reaction (bacteria and plants):

α-Ketoglutarate + glutamine + NADPH + H^+ $\longrightarrow$ 2 glutamate + $NADP^+$ (22-2)

The net reaction of glutamine synthetase and glutamate synthase (Eqns 22-1 and 22-2) is

α-Ketoglutarate + NH_4^+ + NADPH + ATP $\longrightarrow$ L-glutamate + $NADP^+$ + ADP + P_i

So, that is all about nitrogen fixation. So, now from nitrogen we have become ammonia**.** So, now how does that get into the form in which nitrogen exist in amino acids and other molecules? So, that is what we are going to discuss in this slide as well as I think. Oh no, only this slide that is it. The next slide is going to be regulation of these enzymes that we are going to see. So, the first reaction the all the amino group in your amino acids and the nitrogen in other molecules.

They all entered at one point of time into a plant glutamic acid**,** glutamate, this is an amino acid. So, remember this is a carboxylic acid side chain containing amino acid CH 2 CH 2 COH, that is side chain carboxylic acid. And that gets aminated to form glutamine; this is another amino acid, like asparagine glutamine, so aspartic acid, glutamic acid. So, that is how the nitrogen wherever you find in your body originally entered into the biological system**,** the amino group of the glutamine.

So, how does this happen? This we have already seen when we were discussing about ATP, we already discussed, ATP is not freely hydrolyzed from the energy used. Usually an intermediate phosphoryl group transfer happens. So, here the phosphoryl transfer, the ATPs the terminal phosphate is transferred to glutamate forming gamma glutamine phosphate. So, this is now my energy molecule and this one combines with ammonia to form glutamine. So, this is the route by which nitrogen enters into our system.

And this enzyme glutamine synthetase exists not just in plants and microbes, it exist in all animals. So, in many of our metabolic reactions we have not it discussed probably we will briefly discuss amino acid catabolism, we only focus so far only on glucose. So, the catabolism of amino acids end up producing ammonia and that ammonia in different tissues or different organs of our body are converted into glutamine by this very same glutamine synthetase.

And that glutamine is transported via blood to liver and in liver the ammonia is released for other reactions which we will discuss later, probably in tomorrow or day of tomorrow's class. So, therefore this enzyme is an important enzyme, the main thing here you need to remember is, it is present in animals as well, because ammonia production is not just only through the nitrogen fixation.

So, through other catabolic reactions in our body the nitrogens from many molecules are converted into ammonia. And that ammonia is incorporated into glutamate into glutamine and that glutamine is the one that is brought to liver for the excreting ammonia**.** Here in such a context we are talking about already existing nitrogen, so in the root nodules what is happening is a new ammonia is produced from atmosphere free nitrogen**,** so that is the difference.

The other enzyme on the other hand glutamate synthase is only in bacteria and plants. So, this one what it does is, it does a simple transamination reaction, we will discuss transamination reaction in detail when we go into amino acid catabolism. So, all you need to know for now is this, I will just draw that briefly here. If you take like for example, if we take alpha-ketoglutarate, so you have a COOH group and then you have CH 2 and one more CH 2.

Then you have a ketone, and then this, so this will be alpha-ketoglutarate. Now this structure is converted into NH 2 by a reversible reaction with another one having another keto acid. So, through transamination reaction you have this, so this conversion is what we call as a transamination reaction**.** So, alpha-ketoglutarate of which were the double bond O structure, now by transferring amino group from another amino acid.

For example from aspartic acid which would have had a structure like this. Now this becomes amino acid and the other one the aspartic acid would have become oxaloacetate**,** so this is a reversible reaction. So, this is what is a transamination reaction the mechanism of which and the cofactor involvement we will learn in a subsequent class**.** So, such a reaction converts alpha-ketoglutarate plus glutamine you get 2 glutamates because the amino group transfer from glutamine to alpha-ketoglutarate is one glutamic acid.

But after donating that amino group in the side chain this itself becomes a glutamate**,** the side chain is this one, so here it will be COONH 2 to be a glutamine. And after transferring that into alpha-ketoglutarate it becomes glutamic acid like the structure that is drawn here. So, this is a amination that is reductive as well, alpha-ketoglutarate is reduced and this NADPH is oxidized. So, this is glutamate synthase reaction, so the net reaction of both of this if you put together what you find is ammonia is used to convert a keto acid into an amino acid. So, this is how we get the amino acids made by incorporating ammonia which is produced by the nitrogen fixing bacteria from atmospheric nitrogen.

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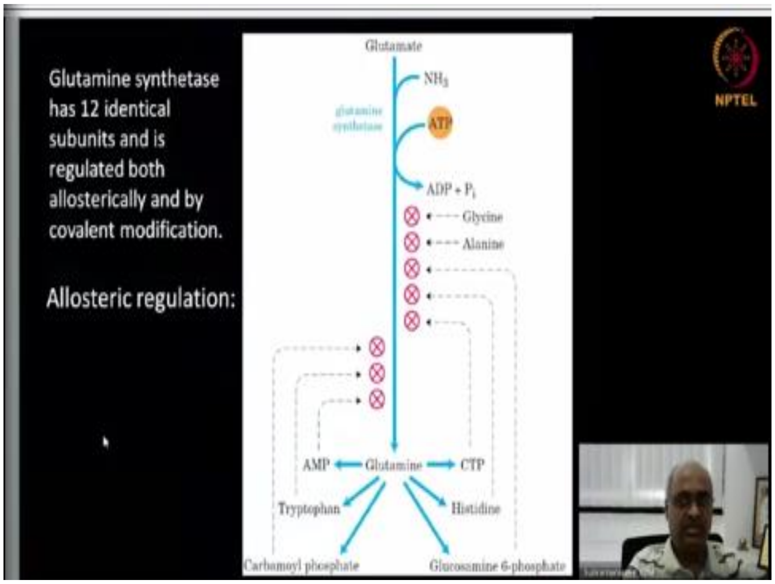

Glutamine synthetase has 12 identical subunits and is regulated both allosterically and by covalent modification.

Allosteric regulation:

So, now given this glutamine synthetase it is central role because this is the one in which ammonia enters into the biological system. It is subject to a really complex and very fine regulation**,** so we will try to understand that. That is the only thing we are going to do in the remaining few minutes today. So, you need to realize all the ammonia that you talk about, all the amino acids the muscle, protein, this and that you talk about.

And really hype about the importance of protein for muscle power all of that, the whole thing enters into your muscle proteins via this enzyme glutamine synthetase. And this is what incorporates ammonia into the biological system in all organisms, due to that this is subject to a really, really serious regulation. And that regulation is what is outlined in this as well as next slide.

So, first this enzyme is subject to allosteric regulation, remaining molecules binding to a site other than the active site altering the catalytic activity of an enzyme. So, it is subject to feedback inhibition by variety of amino acids**,** glycine, alanine, histidine, glucosamine phosphate which is not an enzyme, but this glycosaminoglycan, not glycerin, so this is a single monosaccharide.

Then this nitrogenous nucleotides as well**,** so here you have a cytosine triphosphate. So, all these end product of nitrogen containing molecules, they all act as negative regulators, similarly if you do not have the material to produce this. For example if you do not have ATP and you have plenty of hydrolyzed form of that then that also inhibits it. Because you cannot make this without these and this is the allosteric regulation, essentially feedback regulation by end distant products, not the immediate end product of that reaction.

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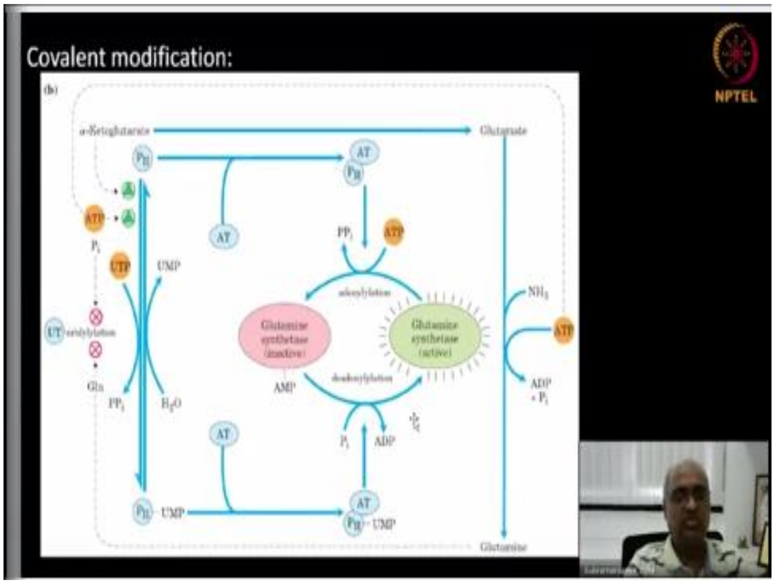

Covalent modification:

And in addition to this we also have a very complex regulation which is not that difficult to understand. This enzyme is regulated by covalent modification, remember when we learnt about enzyme regulation, we learnt about allosteric regulation as well as covalent modification. So, when one of the amino acid side chain is modified, we learnt about phosphor modification with phosphate groups as one of the main regulations, phosphorylation of enzymes.

So, not only just phosphate groups other moieties are added to. In this particular case, it is the adenylate or adenylic acid, adenylic acid is nothing but adenosine monophosphate AMP. Like in ATP if you remove the 2 phosphates and leave only 1 phosphate in the ribose sugar. So, that is adenylate, the ionized form and otherwise it is adenylic acid. So, that is why it is adenylylation is what happens.

So, that group AMP is covalently attached to the enzyme and when that adenylylation happens, it becomes inactive. And this is done by adenylyltransferase that is what is this AT here, so that transfers adenylyl group and that transferase enzyme can do both depending on what is bound to it, it can add the adenylyl group to the enzyme or it can remove it deadenylate it. It is done by the same enzyme AT, it depends on AT is associated with whom.

So, now the active enzyme of course is going to incorporate ammonia into glutamate and forming glutamine and the inactive enzyme will not (()) (36:27). So, now let us look at who is associated with this AT, say a protein called P II when it associates with AT, it activates the enzyme and it inactivates the ammonia fixing**.** On the other hand if the P II itself if it is covalently modified by adding and uridyl group the uridylated version of P 2.

If that binds to AT, it promotes the deadenylation and that would make the enzyme active. So, adenylyltransferase is the one that covalently modifies our main enzyme glutamate synthetase. If it is without the adenyl group it is active, and if it is with it, it is inactive and this is done by an enzyme which can do both the reactions depending on what form of another protein associates with it and that protein is P II.

Now let us look at how P II is regulated? So, what P II does, then we will think about the regulation, right now do not look at all these triangles, circles, green colour all that, only look at the blue. The P II as we see here it can be free P II or UMP P II and that is done by an enzyme on UT uridyl transferase. So, that enzyme is the one that is catalyzing this reaction, that enzyme is subject to allosteric regulation.

So, if it has a lot of glutamine, that glutamine is going to inhibit it, if it is inhibited then you are not going to have P II UMP instead you will only have P II. And P II is going to inactivate this enzyme, because when you have lot of glutamine, why do you want to make any more glutamine**,** so that is what it does. On the other hand, and similarly if you have inorganic phosphate, meaning ATP is not available, most of it is in hydrolyzed form and that also inhibits it.

But on the other hand if you have a lot of ATP and your lot of this keto acid available then you can go ahead and make more glutamine. And they are going to promote this deuridylated form, so like this enzyme AT this UT also does both the reactions**.** So, these are going to promote the reverse reaction and as a result you have unmodified free P II and the free P II inactivates this so this is how this regulation happens.

So, it looks complex, but it is actually not complex you only have 2 hierarchy here; one is glutamine synthetase itself is activated or inactivated by covalent modification. And that covalent modifying enzyme is also similarly subject to activation inactivation by another enzyme that does covalent modification of this ATP. And that enzyme is allosterically regulated based on the molecule substrate or product availability.

When you have products, it is going to end up in this version and that is going to if it is the product of the final this reaction glutamine for example if it is there, it is going to inhibit this. And therefore you have free P II and that is going to inactivate this enzyme, therefore no product is produced. On the other hand, substrates are there that is going to activate this enzyme therefore you have this UMP P II.

That is going to promote the de-adenylation, therefore it becomes active and you will make more glutamine. That is all you need to remember 2 levels of regulation. An enzyme that covalently modifies and that enzyme itself is subject to covalent modification and that covalent modification is regulated by which one is available, whether the substrates or the products. So, this is not immediately relevant for us but the ATP is what finally goes and activates here.

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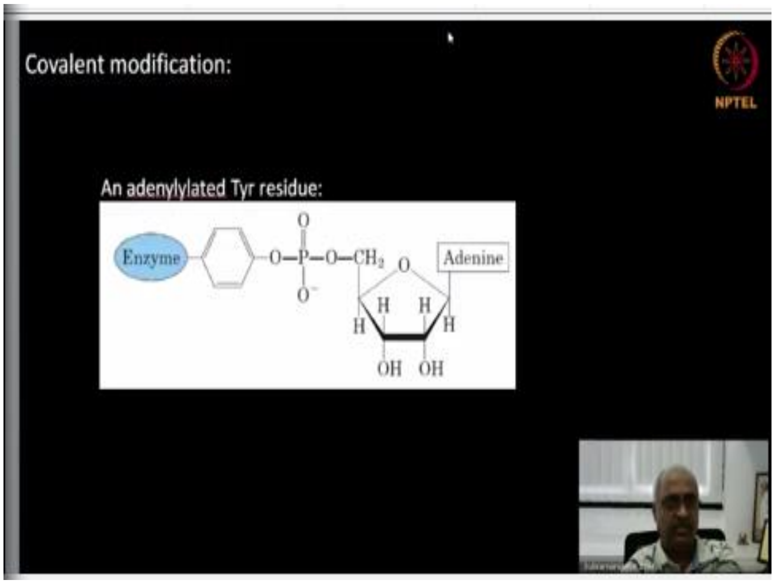

Covalent modification:

An adenylylated Tyr residue:

So, this modification group is shown in this slide. So, this is the enzyme tyrosine residue an enzyme one of the amino acids of the enzyme it is a side chain being tyrosine which is the hydroxy benzoyl group. That is in ester bond with a phosphate and that phosphate is part of this AMP, so adenine, ribose and then single phosphate. So, this ester between AMP and the tyrosine residue of the enzyme is the covalent modification here, this is the adenylylation.

In addition to it now we have seen 2 levels of regulation, one allosteric regulation primarily by the amino acids and the energy availability whether you have ATP or ADP, so we saw that. Second is this complex covalent modification and the third is this enzyme this UT also acts as a transcription activator or inactivator of the glutamine synthetase, gene itself. So, transcriptional activation of glutamine synthetase means you will produce more of the glutamine synthetase mRNA and more of the glutamine synthetase.

Transcriptional inactivation means opposite. So, you have 3 levels of regulation of the glutamine synthetase. It is primarily because this is a very crucial central enzyme in the nitrogen metabolism. So, I will stop here; I am stopping little early today and if you have questions you should use this opportunity to ask questions. And in the next class we go into the amino acid catabolism.

How the amino groups and the nitrogen from purine, pyrimidine etcetera, how they are all removed? Because free amino acids do not exist so much in our system like the way glucose exists, then, see you guys tomorrow, bye.