Biological Inorganic Chemistry
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Lecture 42
Carbonic anhydrase and lyases

Hello everybody. So, a very good morning to all of you. So, we are in lecture number 42, where we will just introduce now where the beautiful biochemical reaction, the zinc 2 plus ion can perform, that means in carbonic anhydrase and lyases. Just simple example of lyases we will keep.

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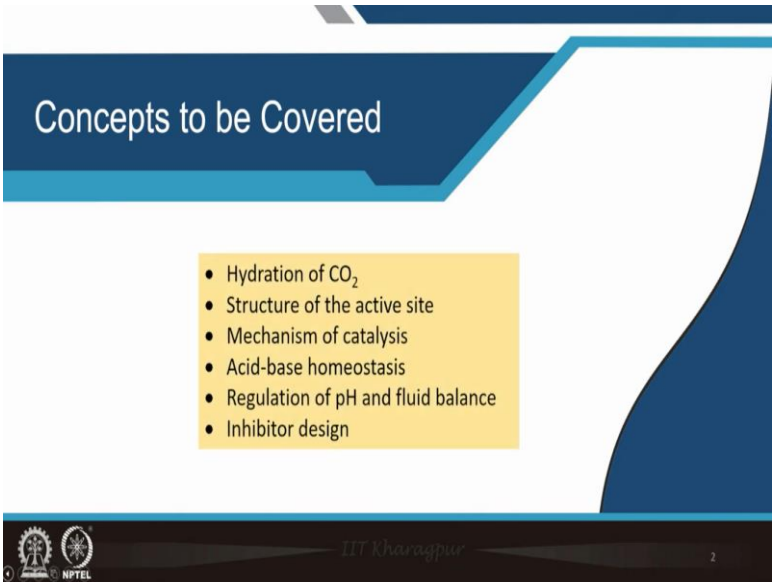

So, the hydration of CO 2, we will see that how it can be done by a particular type of metalloenzyme which is your carbonic anhydrase and there we will find what should be the structure of the active site because always very important how the structure is formed and what particular role the structure is playing.

Then briefly the mechanism of catalysis we will see and all these things are very much related to the acid base homeostasis. So, if we talk in terms of the corresponding hydration of CO 2 and we should also try to correlate how the acid base equilibria or acid base homeostasis is related to that hydration only. That means your proton can be delivered from the hydration reaction where your water molecule is getting attached to your CO 2 molecule.

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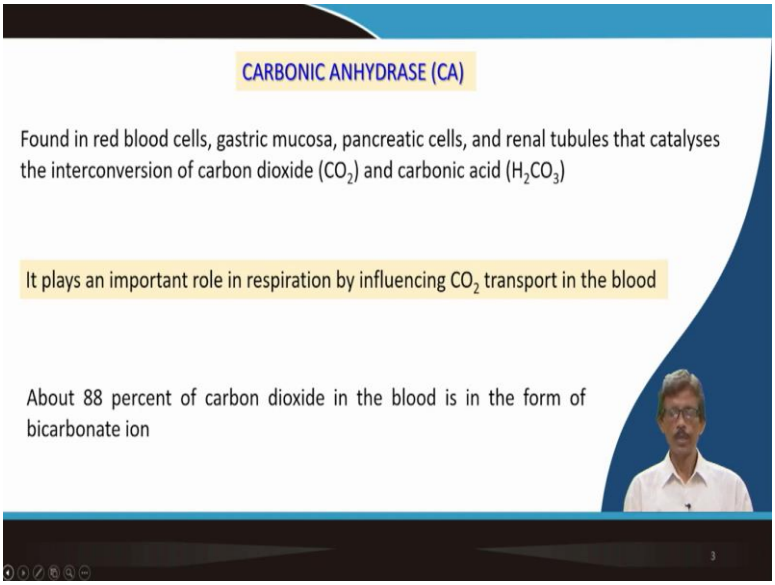

So, we will talk about the internal regulation of our pH in our body and in different biological bodies also, their fluid balance and finally the individual design for stop or inhibit the reaction of carbonic anhydrases. So, we will basically abbreviated as CA, C capital A, as the carbonic anhydrase and we find it in our body also, there we call as the human carbonic anhydrase HCA. So, there are many such varieties available, a large number of these enzymes are working in our body, in other biological system also. So, where it is? It is in gastric mucosa. It is in pancreatic cells. It is in general views.

Basically, all these things are important because it can affect also our kidney, the removal of waters from the system, because these equilibria is involving water molecule, very important water molecule as well as the carbon dioxide what we are producing through assimilation of your breakdown product of your glucose molecule or any other food material, the fat, or the protein molecules, where we have the carbon source.

But the assimilation is done through your dioxygen molecule which we have seen earlier that how we can take that dioxygen molecule from your hemoglobin and myoglobin molecules attaching to that. So, it also there so plays an important role in respiration by influencing CO 2 transport in the blood. So, earlier we have seen that we also explained that how the O 2, the binding of the O 2, the transport of the O 2 is important for our respiration process. Then while seeing the corresponding copper enzymes, we have seen that the electron transfer by these copper enzymes are also useful and finally, we can deliver 4 number of electrons to the O 2 molecule for the working principle of your respiratory enzymes.

So, here the question will come definitely how the respiration can be influenced by the corresponding concentration of CO 2 and its typical catalytic conversion because this conversion is very slow if we go a laboratory conversion for the hydration reaction of carbon dioxide molecule which can be very slow. To enhance the rate of the reaction or the feasibility of the reaction, we take the help of this particular metalloenzyme.

So, definitely, it will therefore be related to your transport of blood and the carbon dioxide converted to some bicarbonate anion or the free carbonic acid can be attached to the peripheral part of your for ring. So, how much carbon dioxide we can store? That means 88 percent of CO 2 molecule in the blood is in the form of bicarbonate ion.

So, here you see that not only we are producing carbon dioxide, but immediately the carbonic anhydrase metalloenzymes are coming into the play that it immediately convert. So, mostly the 100 percent, not even 100 percent, but only 88 percent of the right conversion of your all the CO 2 molecule is converted to bicarbonate or in its other free form that means the free carbonic acid.

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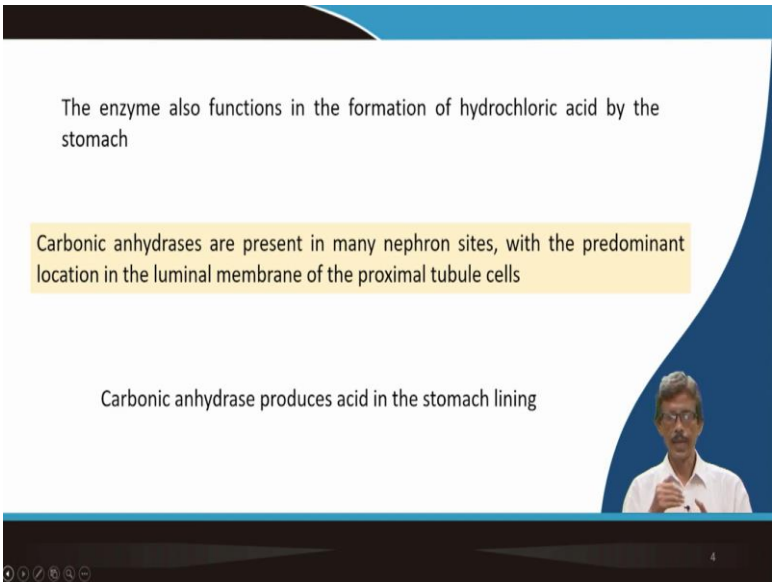

So, this metalloenzyme therefore also can function in the formation of hydrochloric acid by our stomach. How? So, you try to correlate all these things. We have only the carbon dioxide molecule. We are going for the simple hydration reaction and that hydration reaction is nothing but the attachment of that H2O molecule to CO 2. And we all know that CO 2 is a very linear molecule of carbon at the center and at 2 ends you have the oxygen. So, you have to activate that CO 2. How we can activate that we will see.

But here, if the statement tells us that it is also related to the formation of hydrochloric acid in our stomach, we know that the stomach pH can goes down to 2.8 or around 3 only since highly acidic in nature, but we are producing by this particular carbonic acid formation but the carbonic acid pKa is very less. It is not that immediately it will go for the deprotonation. But if you go for the corresponding balance of the carbonate bicarbonate and the free carbonic acid, the equilibrium between these 3 species, we will find that we will be able to produce large amount of protons or large amount of hydrogen ions, and all the time, we know that the corresponding concentration of the chloride ions.

So, if we have some time, and definitely in one module, we will talk about the corresponding biological inorganic chemistry of nonmetallic species like chloride, phosphate, all these. So, there, we will find that the chloride concentration inside the cell and outside the cell is also important, and whenever you have the free H plus produced, it will immediately trap or bind that chloride ion available in that particular environment and can produce the hydrochloric acid.

So, that is why you are able to produce hydrochloric acid in our body in our stomach, but not any others because we have the phosphates, we have the phosphoric acid some part, but we are not able to produce any kind of phosphoric acid in the stomach. So, that is a very beautiful question you should able to understand that.

So, while also it can present, it can present some more sophisticated places like many nephron sites, and is also a predominant location in luminal membrane of the proximal tubule cells. The tubule cells are the cells where we can take up the water while our kidneys producing that water and we can excrete the kidney along with other salts other inorganic salts, but the production of that particular water is related to that of your equilibrium which is being controlled by CO 2 and H2O.

So, whenever your bicarbonate anions and other anions are there, so bicarbonate anion can take up one proton and can eliminate the water molecule. So, the balance in the water molecule also in the system is important. And the balancing of water molecule production and the excretion through kidney is also important.

So, therefore, where it produces, we are producing hydrochloric acid and in the stomach lining, that is why it is very dangerous also sometimes if you have huge amount of acid at the stomach lining, it will also be affected by ulcer, the gastric ulcer, the duodenal ulcer. So, all

these things because the lining will be destroyed for the continuous presence of your strong hydrochloric acid in your body.

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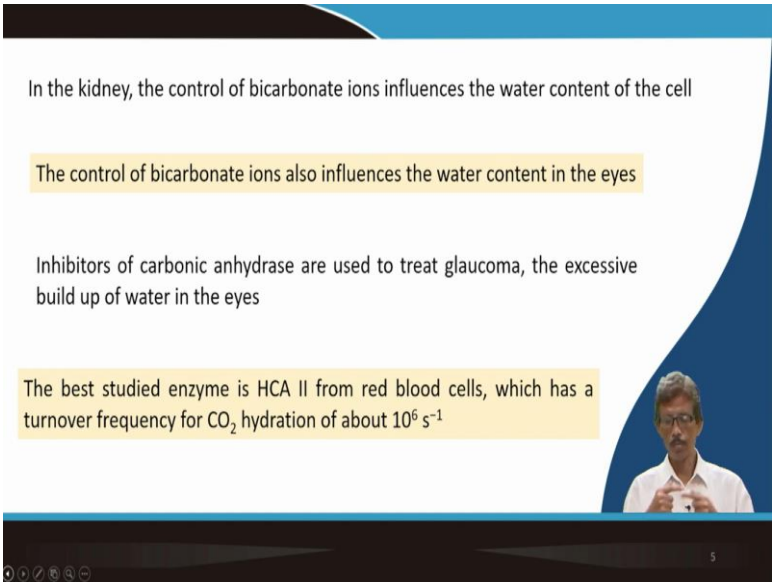

So, this control of these waters, how kidney can take up this water, so the control of bicarbonate ions basically influences the water content of the cell. So, how much water content will be there? We know that the water content in a semi permeable membrane we can think of, we can talk about osmosis. But if we think about the passage of ions like sodium ion passage and the potassium ion passage, but we can talk there in terms of the corresponding charge balance, which is your the chloride balance.

But if it is water balance, though these water balance can be dependent on this particular equilibrium involving carbon dioxide and these water molecules. So, therefore it also control in many other places like the water content, the ocular pressure, the water pressure, how much water we have in our eyes. So, it also influences the water content in our eyes.

So, that is why we will see that one diseased condition we know as glaucoma and the glaucoma is nothing but the internal eye pressure is changing. We can measure the eye pressure. There are instruments which can measure your eye pressure, but the diabetic patients often go for this problem of glaucoma. So, for to treat that glaucoma what you can do? Because this is the overactivity of the CA molecules or the carbonic anhydrase metalloenzymes. So, you should reduce that activity of the CA molecules or the carbonic anhydrase molecule such that you will not be able to produce huge amount of water molecules out of these reactions of carbonic anhydrase.

So, just as I told you, that is the human carbonic anhydrase of type variety 2 because we can have alpha, we can have beta, and we have gamma, delta, epsilon, and all. The different sorts of these carbonic anhydrase. Large number of carbonic anhydrase is unknown in our system and this particular case, which is well studied because for the during the last 30 years or so, the studying for the theoretical chemist, the experimentalist, the biologists, they all are studying on these HCA 2 to find out basically the biophysical measurements experimentally as well as theoretically tells us that it has a turnover frequency about 10 to the power 6 second inverse.

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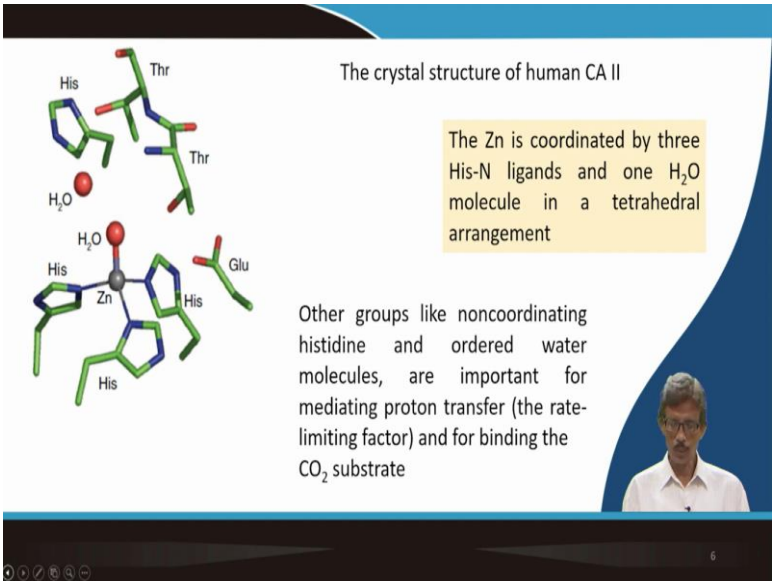

So, the very turnover frequency, so the catalytic cycle is also in a very fast rate it is giving the cycle and we are able to produce all these things. So, coming back to the corresponding assembly, what we can have the structure. So, you see now, what is your HCA 2. So, HCA 2, so we have considered it because in this particular module we are talking about the metal ion zinc 2 plus.

So, definitely, if somebody asks you that what is the crystal structure of human carbonic anhydrase 2? So, immediately your answer would be that I know that it has the zinc center and zinc center we are talking about the corresponding hydration of the carbon dioxide molecule. This carbonic anhydrase or carbonic acid dehydration something like that. The equilibrium we are trying to talk about.

So, you have the zinc center and how the zinc center is located inside the protein pocket that we can see. So, if you have the huge structure is given to you, only the active site or the zinc

ion site part we will find that you can find out what you are immediately find out the presence of the zinc ion at the center of tetrahedral coordination geometry.

And that tetrahedral coordination geometry is formed out of the 3 of these always we say this is a very useful coordination geometry for the biological world is a facial coordination 3. My 3 finger tips basically, 3 of these basically, coming out from the histadine residues. Though histadine immediate groups. Immediate is the pendant group and that pendant group from the amino acid is used, is available to coordinate your zinc center.

So, that gives you a try coordination to the zinc center initially. Then zinc is binding over there. So, that is the strong binding basically that is why yesterday or in a previous class where I told you that you can have the bowl step thing. So, if you have the bowl, on the bowl your zinc is sitting and above it your water molecule will come because no other molecule is available to bind or satisfy the fourth coordinates inside of the tetrahedron geometry.

But zinc will have the affinity always to satisfy this coordination number because it will not be happy with the coordination number of 3. So, it can go to a coordination number of 4 and when this water molecule is binding, you will have a definite zinc oxygen distance the the zinc ion oxygen distance when you go for the crystal structure determination, we can find out that particular distance.

But other 2 things also we try to remember because it's not that you when you determine the structure about the zinc and the zinc environment, it is not that it is everything you know. We try to find out the other water molecule because that water molecule is important in transferring these protons and transferring these hydroxide ions because this will be informed during the reaction of your CO 2 molecule.

So, if you consider that no this water is directly forming a coordinate bond to the zinc center, the second water molecule towards the left but we are not showing any bond. It is there. If it is a very good crystal, we can talk about the crystal lattice water molecule where we write the center dot H2O for the different formula of the different molecular salts or the different metal ions salts like your simple copper sulfate or the copper acetate.

Here also, the protein can have one structure like that, but it is not like that. It is bound much strongly by many other things like that of your hydrogen bond that will be on the coordinated water molecule, then you can have the histadine residues nearby. That histadine residue will

have we will see a very important role it will play as a good base because the histadine tertiary nitrogen, not that NH nitrogen. NH you have another is histadine.

So, that it can take out the proton and it can function as a very good base. Then 2 other amino acid residues or the another part basically, the dipeptide basically is important and is available around this particular one which is covering this particular active site. So, that is why what we have seen is written over here. So, we have these, all these. So, what are these non-coordinating molecules available around these active site?

So, a coordination chemist always thinks about the environment, what the metal and can give you and the definite metal and bonds and other things. But when we go beyond the coordination, that means the supramolecular or supracoordination, because the beyond the coordination thing, we can have something. Its not that all these are going away.

Since zinc is present, since zinc bound water molecule is present, all other groups will be arranged over there for a good type of hydrogen bonding interactions with this coordinated water. So, if more number of water molecules are coming, we can have ordered water molecules and which are important to mediate something which is very interesting in the biological world. That is the proton transfer.

So, this proton transfer is a very important thing and most of the time when we talk about the electron transport, even in the batteries also, the fuel cells and all these things, we also talk about the proton capture and the proton transfer potential and all these things because this is a rate limiting factor. Until and unless you go for the deprotonation and the protonation steps, you cannot see the corresponding catalytic activity of this particular site, which is responsible for the binding of your CO 2 molecule.

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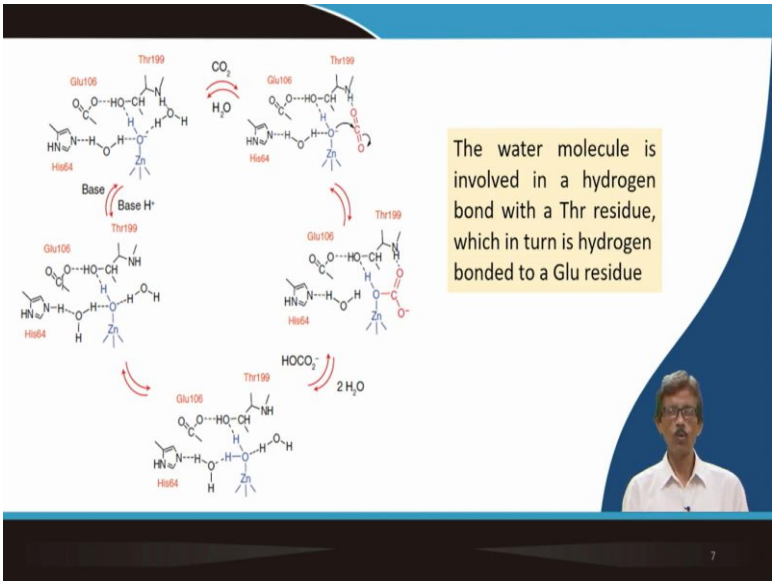

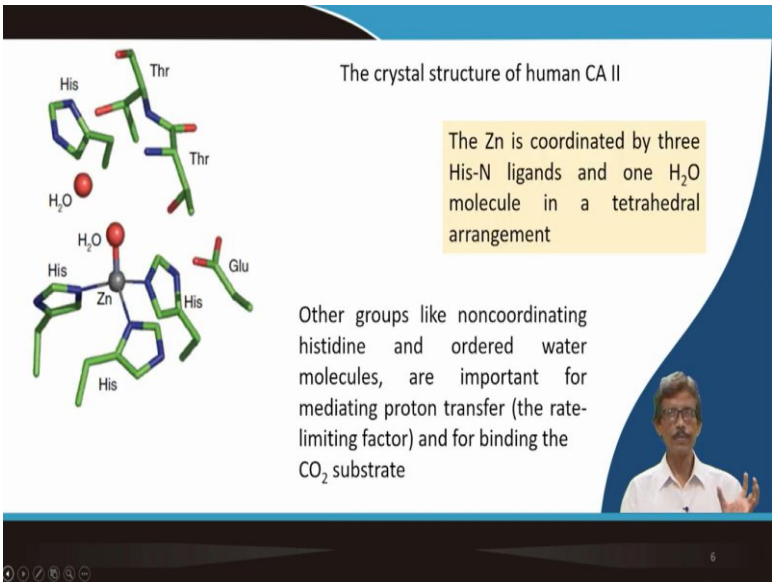

So, altogether, if you assemble all these, now what you have to see, because this is the site and now we will only talk about the zinc site and the top environment, that means the umbrella part what we know that is you are covering with something which is your umbrella part. So, that umbrella part how it is organized. So, you just start with that off your top left.

So, top left is that structure what we have seen just now, you have the histadine. Now it is numbered. So, histadine, the glutamate, the threonine, and all. So, if you have not only the 3 amino acid residues from the polypeptide chain coordinating the zinc, but also you have 3 other amino acid residues which is basically giving you an envelope such that you can trap more number of water molecules around the zinc site where you have the zinc bound or coordinated water molecule.

So, you have something in the left and something on the right also. So, you have a water molecule trinucleid. If 3 oxide water molecules you are able to detect that means that trinucleid water cluster is there. One is bound to the zinc center and 2 are nearby. So, then the next step what is happening that you get for that you are losing some hydrogen bond interactions when the CO 2 is coming and that introduction of the CO 2 is destroying one water molecule to be attached at the hydrogen bonded form. And that basically goes for your thing, that when it is going away, it goes in a way that it is taking that particular case that means is a linear molecule.

So, CO 2 how CO 2 is coming close to that of your zinc center because the zinc bound water molecule or zinc bound hydroxide ion through deprotonation, you can have the zinc bound hydroxide ion can be a very good metallion activated nucleophile. So, that metallion activated nucleophile now will attack on the CO 2 molecule.

So, we have seen earlier also, today we are also seeing that the nucleophile will attack the carbon center, the carbon atom of the CO 2 molecule because you have the charge polarization because you have the electronegativity difference between carbon and oxygen. So, there will be a charged polarization. So, carbon center is the positively charged center, delta plus and 2 oxygen centers are delta negative sites. So, then you have, if you have, you have the attack of the CO 2 molecules, a linear molecule, you are able to attack by the nucleophile. So, what will happen?

This particular one, this site which you can have, so it can just go for the bending. So, CO 2 molecule we try to bend because we know that the CO 2 molecule can have the corresponding stretching vibration, the bond stretching vibration as well as the bond building vibration.

So, in one such bending vibration, so your voice will come and attack that particular carbons to the nucleophilic attack on that particular carbon is trying to give you a corresponding bond formation which is that means a new carbon oxygen bond can be formed over there. But the involvement of another water molecule is important in such a way that when we have these C because the other end of the CO, because the CO can have the lone pair of electrons and which is nearby of your zinc site.

So, on the top right, you will see that the lower oxygen of the carbon monoxide molecule is close to that of your zinc center and it gets some activation because the lone pair electron density can be partly donated to the zinc ste such that your oxygen can function as a base. So,

this particular bending and the attack of all these things can give rise to sometimes a very closed or six membered metal cyclically ring involving zinc, involving your hydroxide ion, and involving another water as well as the CO bond.

So, CO bond is a double bond. The OH of the zinc is also a double bond and another OH of the water molecule is the double bond. So, if you are able to make a cycle out of these 3 species that I want 2 atom species, we will have a hexameric unit. So, that hexameric unit, when it goes, one proton will be lost and another new proton will be attached to the oxygen center.

So, ultimately what you get, we get something where you can have the corresponding species is forming as your corresponding carbonate species. So, this particular one, basically when we have, you put that particular one as your carbonate species, so the carbonate species will be formed around the zinc center and that zinc center now you can have the corresponding hydrogen bonding interactions. And hydrogen bonding interaction can be re established once again.

So, the simple reaction is that your zinc is giving you the activated one, activated hydroxide ion. Hydroxide ion will attack your CO 2 center and that CO 2 center with the bending form going into the carbonate as well as the bicarbonate species, so HOCO 2 will be forming and CO 2 minus will be forming over there. But the protection from the secondary coordinate are attractions for these water molecules. They are involved in hydrogen bond which THR residue, the threonine residue, and the glutamate residues which are important there.

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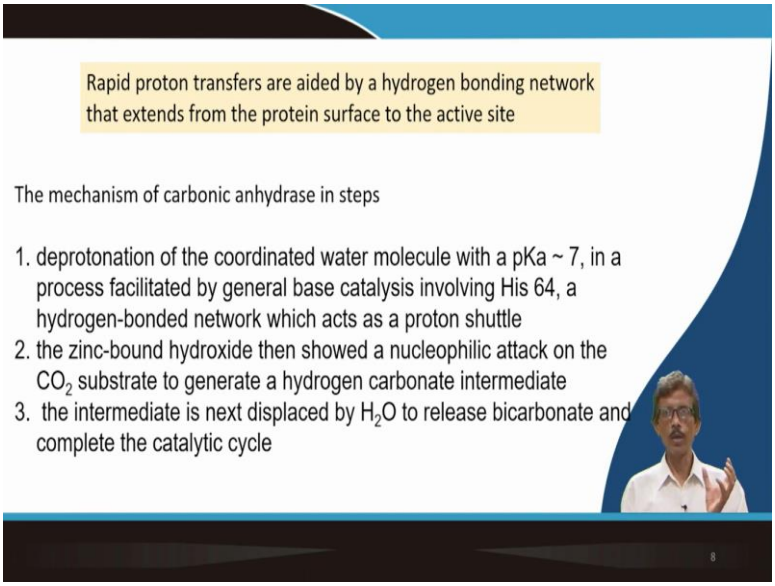

So, what we can see that the chains are there involving zinc center, zinc bound water molecule, another water molecule and the CO 2 molecule. So, you can have a hydrogen bonding network because the carbon dioxide can also be involved in hydrogen bonding network, because it can have some hydrogen bonding affinity not only for your coordination affinity to the zinc 2 site but also it can have some affinity for your water molecule.

So, if we can now discuss all these things, all these steps, basically in terms of the mechanism of the steps so we can have 3 different steps. First one is nothing but the deprotonation which is important because all these languages will be with you, do not worry to read it now, you read it afterwards. That is why I am giving is the material as a starting material to you also, that when we know that the water molecule when it is the free water molecule, but since pKa, so that pKa of water molecule is about 14, the free water molecule.

But when it is bound to a metal ion, say zinc center, not iron, but zinc center, you drop down the pKa value to a particular level of 10, but when you bring the amino acid residues of the protein chain or the protein polypeptide part, your pKa value is further decreasing. So, you can have a pKa value of 7 and which basically go for the corresponding base catalysis because if you have some inbuilt base is available like histidine 64 which can abstract one of the proton of that bound water molecule on the zinc site and giving you the hydroxide ion on the zinc site.

So, that will facilitate the deprotonization. Your pKa is dropping as well as the base is available nearby. So, you get a zinc bound hydroxide ion and that already I told you what is happening there which will be attacking your CO 2 molecule and the intermediate is next displaced by another water molecule to release the bicarbonate ion and complete the catalytic cycle because the third step, the water molecule, which is coming to kick out the bicarbonate anion is again sitting on the zinc site.

So, zinc is basically dictating again the binding of that water molecule. So, these water molecules are getting changed. So, you should look at all these cases which proton is coming from what water molecule, whether the bound proton of the zinc center is donated to that of your carbon dioxide molecule. It is not that. Its not as such a simple reaction what we write as CO 2 plus H 2 O is equal to H 2 CO 3.

So, that is not the very simple reaction. You have not had some other water molecule and isotopic labeling experiments and many other analytical techniques can tell us that these

water molecule is not fully giving 2 of these protons or one of these protons to the bicarbonate which is formed.

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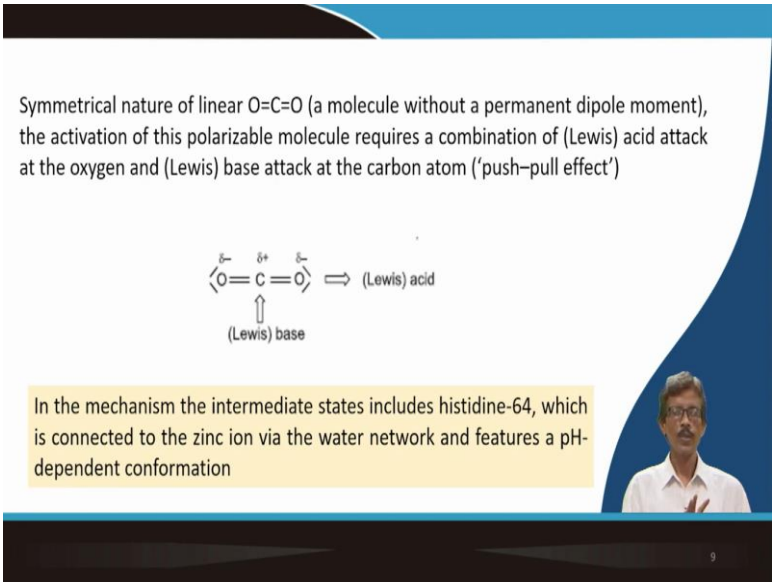

So, you have the linear CO 2, already I told you, we are having a permanent dipole moment because you have the corresponding charge separation. So, delta delta negative. Small delta negative negative on the oxygen and 2 delta positive on the carbon center. So, you have the polarizable thing and that is why we can have a combination of lewis acid attack at the oxygen and a carbon attack by the corresponding nucleophile.

So, as a result, we have the corresponding push pull effect. So, you have the carbon dioxide molecule and you get the corresponding one as your typical push pull effect. So, you have the lewis acid part and the lewis base part. So, you should be able to consider all these things and the involvement of the histadine 64 is important for your change in any type of bond formation because the protein residuals or the corresponding amino acid residues are important to give you the corresponding 3 dimension structure.

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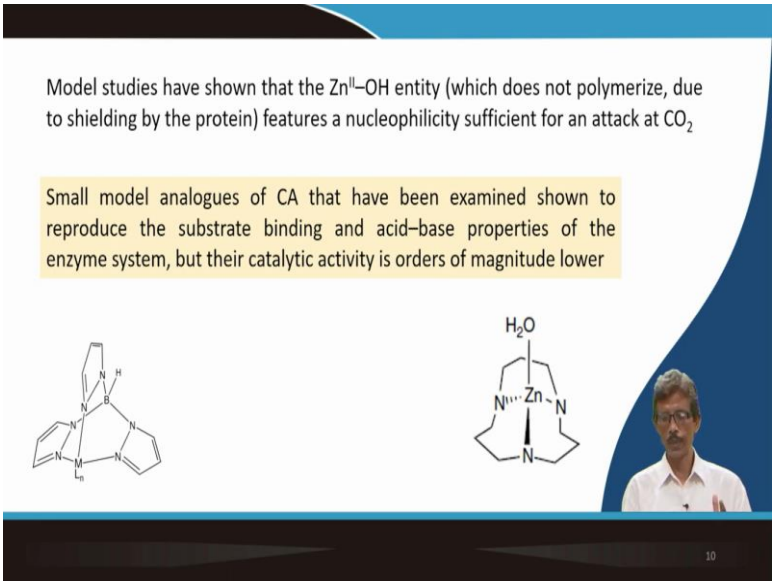

Now, to get these models, how fast this reaction is, what is the corresponding rate of these reactions? We can go for the simple modeling to take the corresponding ligand systems, simple ligand systems. So, small model analogs of CAs are available to check out the corresponding catalytic activity. So, one such is the macro cyclic.

So, is a tridented nitrogen bearing macro cyclical Ligand. So, the space is the corresponding, probe and space are not the ethylene space. We know this one is the triada cyclon variety, that is the bigger variety of that, and you have the zinc coordinated form. And you can have the trispyrazolylborate we all know. That is again a facially caping ligand.

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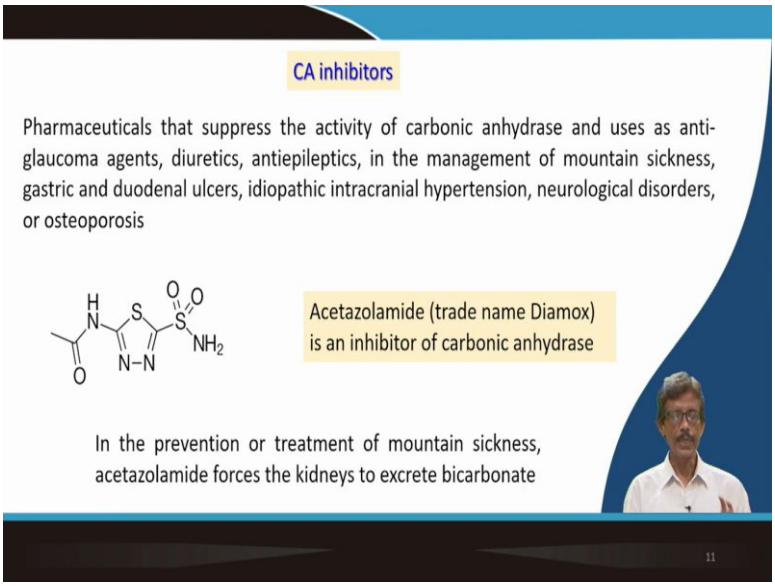

So, that can give you this particular binding to the zinc site and we can study all these things. Now, as we have discussed at one point that you can inhibit the reaction, the enzymatic reactions of carbonic anhydrase. So, how to inhibit that? So, we must have the designing thing, the designing principles.

So, we can have the pharmaceutical productions or pharmaceutical molecules which can inhibit the rate of the reaction or even the reaction for carbonic anhydrases to get antiglaucoma agent which is the pharmaceutical, which is a drug molecule, which is a diuretics earlier we are using but now we are slowly discarding this thing. Doctors are not prescribing it as diuretics as the water balance in our kidney, in our other part of the body, antiepileptics and in the management of mountain sickness which is a very important thing because how much carbon dioxide you are producing is dependent on how much oxygen you are taking.

When you go up in the mountain, your oxygen pressure, partial pressure is less. So, you are not producing many amount of carbon dioxide. But if you retain some amount of carbon dioxide in your body, your body is having some sensation that oh I am happy, I am getting the right amount of O 2, but it is not that.

So, the removal of carbon dioxide molecule can be inhibited and such that the mountain sickness can be stopped, then the gastric and duodenal ulcer to stop the production of H plus, idiopathic intra cranial, the hypertension also, one form of hypertension, the neurological disorder and osteoporosis. All can be tackled by the use of acetazolamide which is a trade name is Diamox and is a inhibitor of carbonaic anhydride.

So, sulphonamide type of drug basically, and we have a heterocyclic ring and that heterocyclic ring, that nitrogen basically, the heterocyclic nitrogen can go and bind to the zinc center to inhibit this particular one. So, it can stop also to use the kidneys to take out the bicarbonate from our body.

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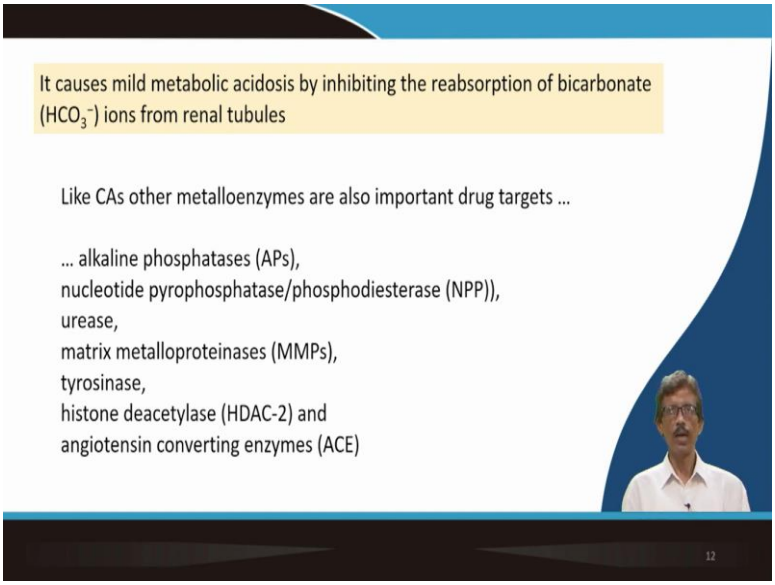

So, it causes also mild acidosis, inhibiting the reabsorption of bicarbonate ions from the renal tubules. So, the CAs can give us a very good idea to study and to understand many other metalloenzymes which can be the target for the drug development because we study medicinal chemistry in a nicer way, but how, why we are studying these metal ion chemistry or the coordination chemistry or the bio inorganic chemistry?

Because we can go for again the alkaline phosphatase to that of your Angiotensin Converting Enzymes ACE. So, a group of molecules written over here, those are all metalloenzymes but more sophisticated metalloenzymes we are not studying here, but you should know the same principle of designing inhibitors, you can apply for these cases.

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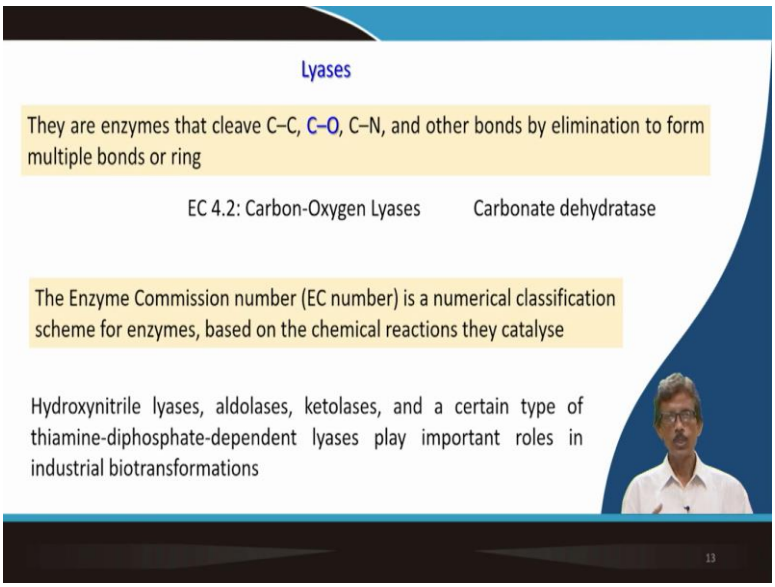

Then lastly, the lyases, what is that lyase? Lyase is nothing but how you can break the CO bond, CO bond of the bicarbonate anion or the carbonic acid to produce carbon dioxide. So, we can have some EC nomenclature for this particular breaking of carbon oxygen bond which we also know as carbonate dehytrase, the hydratase.

So, you just try to say all these dehydratase, so you take out that particular OH part which we have already attached to your carbon dioxide molecule. So, you have the numerical classification based on the chemical reaction and on which particular case, (we) your lyases are falling there.

So, there are many more complicated molecules or complicated parts or enzymes are there which are involving your hydroxy nitrile species, then is hydroxys OH NH 2 species or hydroxylamine we know, the keto function and all other functions, then thiamine diphosphate dependent lyases are there which people use for industrial biotransformations. So, if we can have a good enzyme, we can go for this hydrolysis reactions.

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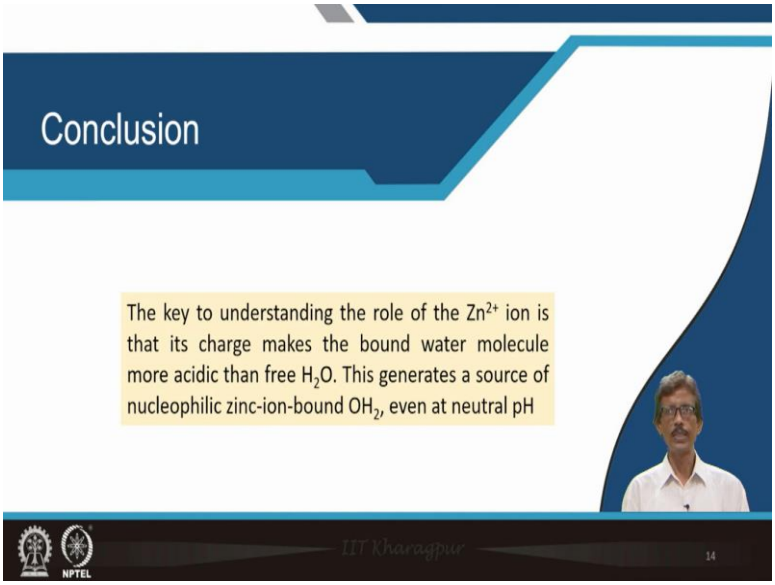

So, altogether, what we have seen here is that the understanding the role of the zinc ion and making some bound water molecules and that water molecule if it releases the proton, it will go or contribute for the acidity of the medium. And therefore, we can have the nucleophilic zinc ion bound water molecule, even at neutral pH because their pH is close to seven.

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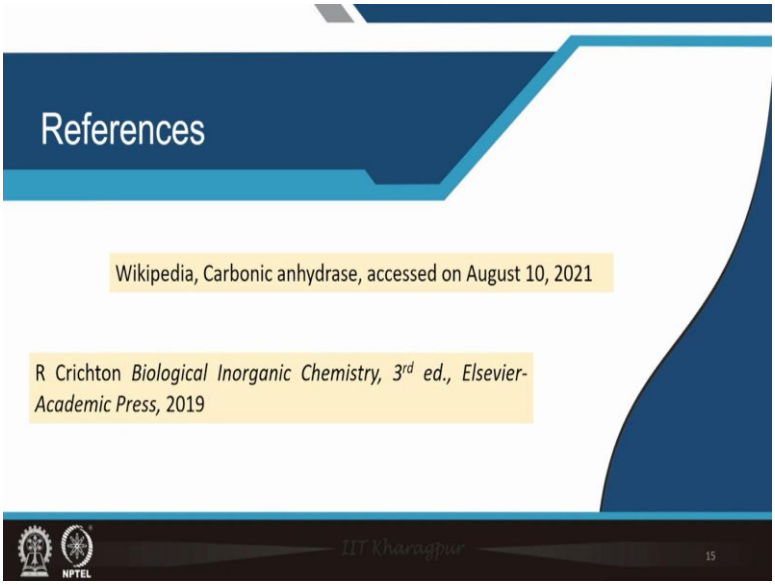

So, you go to the page where you have the carbonic anhydrases we start with, you discussed in the wikipedia page, and the book, every day we talk about this book and every time we consult this book, so you have the book also. So, thank you very much for your kind attention.