Organic Chemistry In Biology And Drug Development
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Lecture – 47
Inhibitor Design of Angiotensin Converting Enzyme

Welcome back. In the last session, we ended up by saying that the Angiotensin Converting Enzyme is a very good target for inhibitor design and if you can design an inhibitor with appropriate properties which include ADME properties and PD properties, toxicological properties, then you can have a molecule which can be sold as a drug, but the molecule has to pass through the clinical trials.

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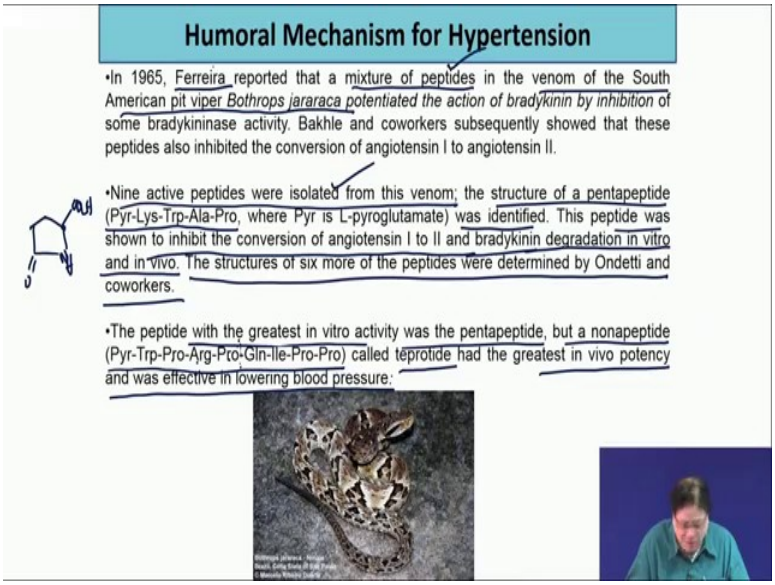

Based on the carboxypeptidase A structure, it was assumed that ACE will have a very similar kind of active site geometry except that it is a dicarboxypeptidase. So, we have to shift the zinc which is binding to the carbonyl and assisting the hydrolysis, little bit away from the carboxy-end. I have shown that this the rough sketch of the active site geometry.

Now, I ended last time by saying that you need a clue from somewhere about your starting point. In 1965, one person named Ferreira, reported that a mixture of peptides from venom of a South American pit viper *Bothrops jararaca* was obtained. It is a deadly poisonous snake. So, from the snake venom, this person isolated a mixture of

peptides and they isolated nine active peptides from this venom; out of these, the structure of a pentapeptide Pyr-Lys-Trp-Ala-Pro; Pyr is what is called pyroglutamic acid. So, glutamic acid which is cyclic, this is what is pyroglutamic acid.

So, it has strangely got a pyroglutamic acid. So, pyroglutamic acid, lysine, tryptophan, alanine, proline. This pentapeptide was identified. The pentapeptide was shown to inhibit the conversion of angiotensin I to angiotensin II and bradykinin degradation *in vitro* and also *in vivo*. So, this was your starting point, the clue was obtained from the venom of this snake and they isolated the peptide.

Nine peptides were isolated and out of this, a pentapeptide whose sequence is shown here was found to be very good in inhibiting the conversion of angiotensin I to II; that means, vasoconstriction will go away and it also does not destroy the bradykinin, causes less degradation of bradykinin, which is a vasodilator. The structures of six more peptides were determined by Ondetti and coworkers.

Now, the peptide with the greatest *in vitro* activity was the pentapeptide as I already mentioned. They also isolated a nonapeptide which is having this type of sequence Pyro-Trp-Pro-Arginine-Pro-Gln-Ile-Pro-Pro; called teprotide had the greatest *in vivo* potency and was effective in lowering a blood pressure. So, lots of peptides were isolated and they found that some of these peptides have very good potency in inhibiting the angiotensin converting enzyme and thereby reducing the problem of hypertension lowering the blood pressure.

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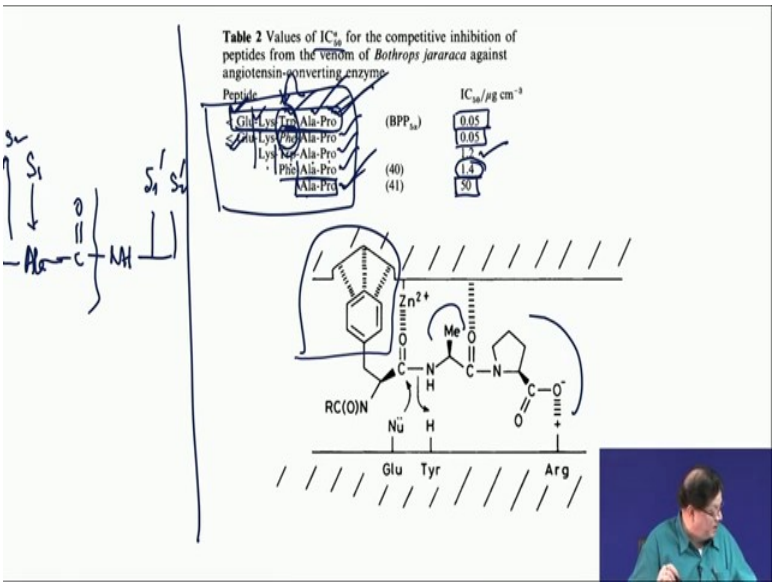

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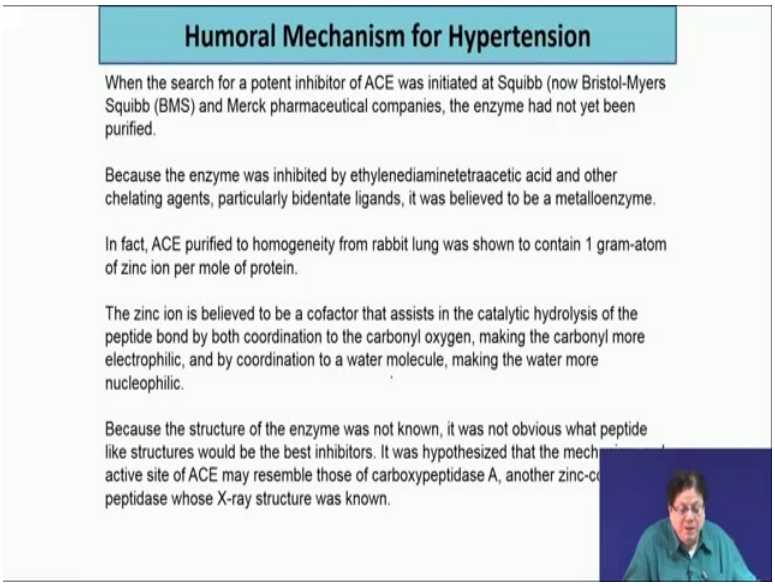

However, these peptides are cannot be drugs because of the problem that before they reach the target, they will be hydrolysed by many peptidases that we have which are non-specific. So, they hydrolyse the peptide before it reaches the target. So, peptides are very difficult to be utilized as a drug. So, although you know that these peptides have very good activity, but you cannot use them in practice.

Now, let us again come back to that pentapeptide glutamic acid-lysine-tryptophan-alanine-proline. IC_{50} is the concentration used to lower the enzyme activity by 50

percent; the amount that is needed to do that is called IC_{50}. So, they are making this type of peptides which are very similar to the isolated pentapeptide because if I go back to the earlier one, you see they all ended with proline at the terminal end.

So, they did not disturb that much, what they have done is only instead of pyroglutamate, they are putting a glutamic acid. So, this pentapeptide has a IC_{50} value very good 0.05 µg/cc and then instead of tryptophan, if you take phenylalanine here, then also the value remains the same. That means at this site if there is a binding pocket that recognizes only aromatic amino acids like tryptophan and phenylalanine.

So, then they started this structure activity relationship. They are slowly taking one amino acid off based on the first peptide. Like the glutamic acid if you remove so, you get lysine-tryptophan-alanine-proline; now the IC_{50} value got increased a little 1.2. That means, glutamic acid gives a better one, but if you take IC_{50} value is increased means actually it is becoming less potent the value is 1.2 that is still not too bad.

And, then if you remove the lysine, and you have phenylalanine-alanine-proline that has got IC_{50} 1.4; that means, there are some minor roles of this glutamic acid, of this lysine and these two. So, it becomes 1.4, but when retain only the last two, you get alanine and proline, you see the IC_{50} value 50. So, IC_{50} value has increased to a large extent. So, this a very bad inhibitor. IC_{50} higher means less potent inhibition.

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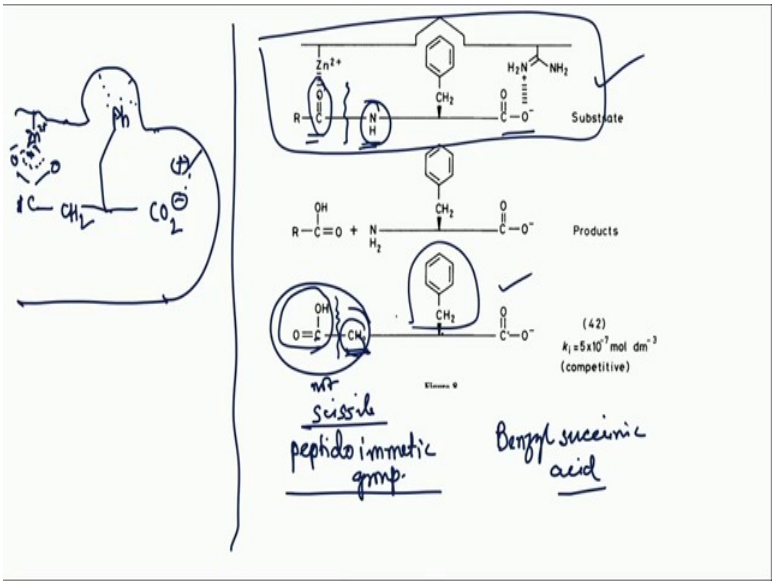

Carboxypeptidase recognizes the aromatic amino acids at the end, although it hydrolyses aliphatic amino acids at the end. So, if I take the aromatic amino acid say phenylalanine carboxy then an N-H and then CO_2 I think I gave you this active side geometry earlier.

So, I start from this carboxypeptidase. So, this is a substrate as shown. Now, this substrate is hydrolysed; that means, the carbonyl is hooked to the zinc to activate this carbon, the phenylalanine has a hydrophobic pocket here. So, that goes and binds and the carboxy must be binding to a plus charged basic amino acid. In this case it is arginine that is for carboxypeptidase. Now, someone found that if you remove this NH and forms a CH_2 here and also remove the carbonyl and instead you put the carboxylic acid here. That means there is no peptide bond after this and this peptide bond is replaced by a CH_2, COOH.

So, what will be the effect of this? First of all this is a sessile bond means which can be broken, it was earlier a peptide bond, but now what you have done, instead of the peptide you have made a carbon-carbon bond which sessile; sessile means you cannot break this. Now, this is what is called a peptidomimetic means you are not having a peptide, but peptidomimetic group. Peptidomimetic group is used just to stop the hydrolysis.

Now, this was found to be a very good inhibitor of carboxypeptidase A. Why it is a very good inhibitor? It has got the CO_2 minus. So, that is bound to this positive charge by electrostatic interaction or a salt bridge and then you have this phenyl group. So, the phenyl is having stabilizing interactions here and then you have a CH_2 and then you have a CO_2 minus; that means, in the biological system it will be present as a delocalized CO_2 minus. So, I can say a delocalized negative charge.

So, that will happen, actually in this position there is this zinc plus. So, now, that will form a chelate to the zinc ion. So, basically you have replaced the carbonyl with a CO_2H. The CO_2H is now ligating to the zinc; that is well known like say EDTA you have this CO_2H. So, it chelates to the metal ions. So, you can have chelation to the zinc instead of NH you are putting CH_2. So, no hydrolysis will take place. This phenyl is still there, that binds to the pocket and the CO_2 minus is also here that binds to the pocket.

So, this is how what is the mechanism of this benzyl succinic acid which is a very simple compound; benzyl succinic acid because this is not phenyl alanine, because you do not have NH here. So, succinic acid is $CO_2HCH_2CH_2CO_2H$. So, you have benzyl succinic

acid. So, it was found that benzyl succinic acid is a very good inhibitor of carboxypeptidase.

So, now from these observations, that these peptides from the snake venom are very good inhibitors and this benzyl succinic acid is a very good inhibitor of carboxypeptidase. So, that gave the scientist a clue how to design an inhibitor of this ACE enzyme. So, what they did, let me again show you. Interesting point is all that for these peptides, the C-terminus is a proline and and then it is alanine; that means, these are the obligatory amino acids if you want to design an inhibitor of ACE.

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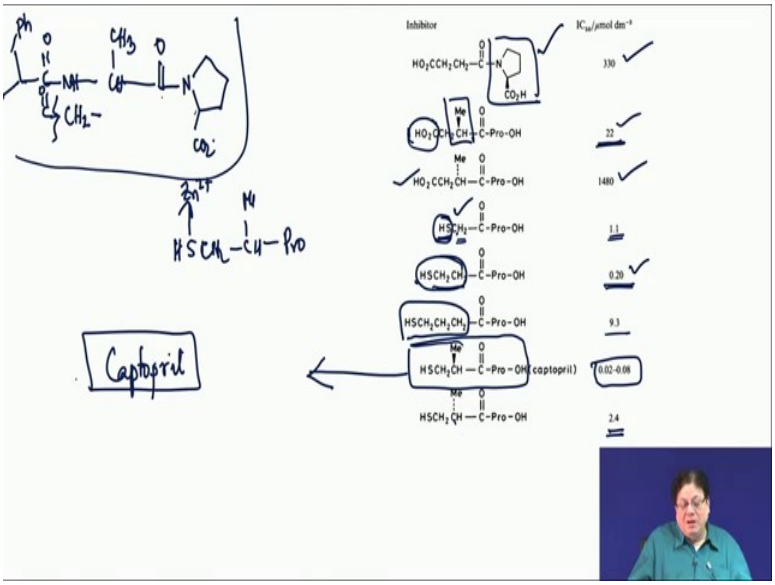

So, what people have done? they made lot of derivatives of proline; like this is a proline; and then you have an alanine; that means, N-CO-CHCH$_3$, this is alanine and then remember if there was an aromatic amino acid here CH and then NH, then CO then there is an aromatic amino acid like tyrosine or the phenylalanine that is in the peptide that were isolated from the snake venom.

So, if you based on this; that means, here you want to make an inhibitor, you should not change the proline. Because all the peptides ending up with proline as the C-terminus, you should not change the alanine also. What you should do, now that benzyl penicillin has given you the idea that there should be CH_2. So, that this cannot be broken now it is a carbon-carbon bond.

Now, based on this, people have started working on that. What you have to do? You have to make a proline, then you have to take an alanine and then take $COCH_2$ then CH_2; that means, a succinic acid derivative here and then you have that phenyl group that should be the ideal. So, people started working on this.

First they had this proline, then CO as I said then they did not take the alanine. They first thought that methyl may not have much effect. So, removed the methyl and then a CH_2 and CO_2H this was the first inhibitor. This was your starting point, they saw that IC_{50} value is very bad 330. Then they started putting the methyl, they put the methyl here. Once they put the methyl it becomes 22; that means, methyl is very important and the stereochemistry is also very important.

If the methyl is α, it has to be L-alanine like stereochemistry; it is not alanine, it is the succinic acid derivative, but in I am just comparing with alanine. So, basically when the methyl is β you have activity, when the methyl is α, have IC_{50} value 1480. So, that means, the stereochemistry is so important which is absolutely required for any enzyme inhibition or enzyme catalysis. So, up to this point you cannot change now, then you have CH_2CO_2H. So, you did not have any peptide bond here, this CO_2H is the one which chelates with the zinc that was the idea.

Then they removed the methyl and then added a sulphur here because we know that sulphur also is a group which can have a very good chelation to the zinc. So, they started putting sulphur. So, first 1 carbon sulphur, then 2 carbon sulphur, but they did not have the methyl yet. So, you see the values slowly going down as they put the sulphur 0.20, before that 1.1 when one carbon spacer is there; then 2 carbon spacer is there, they found IC_{50} value 0.20.

So, first they optimized the number of carbon atoms attached to the sulphur. One is 1.1, two is 0.2, and three is again 9.3. So, that means, these two carbons is the one which you require and then they put the methyl now. So, then they put the methyl and this is the compound and this has got a very good IC_{50} value 0.02 to 0.08; this is what is the drug. And, if you make alpha methyl again as expected it will go up. So, this is the one compound which was first which was approved by the FDA and this compound created a lot of interest in this type of research.

And, you see, this is the first example of what is called the rational drug design because everything started from one clue and then comparing with the existing information and slowly by its structure activity relationship, they ultimately came out with a compound which is called captopril. So, captopril is the first ACE inhibitor which was introduced into the market in the 80s.

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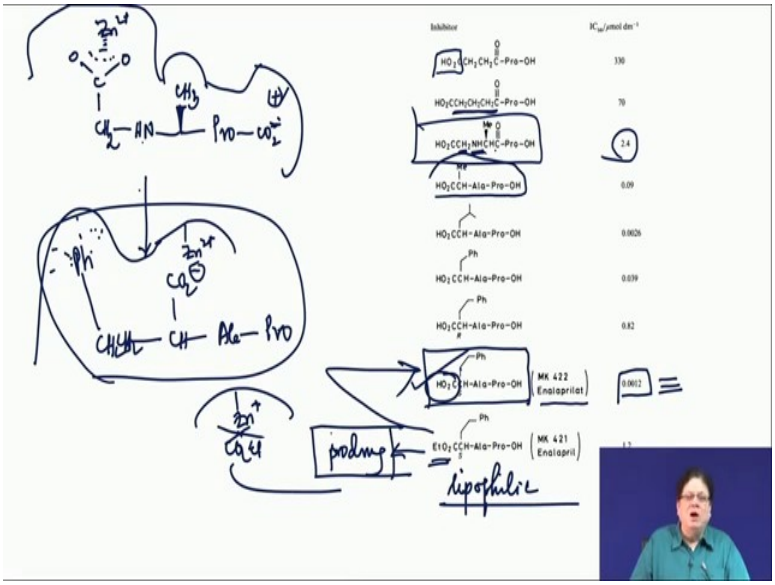

However, that is not the end of the story. Every drug may have some side effects. For captopril, the problem is the sulphur; because of the sulphur, it creates lot of problems. What are the problems?

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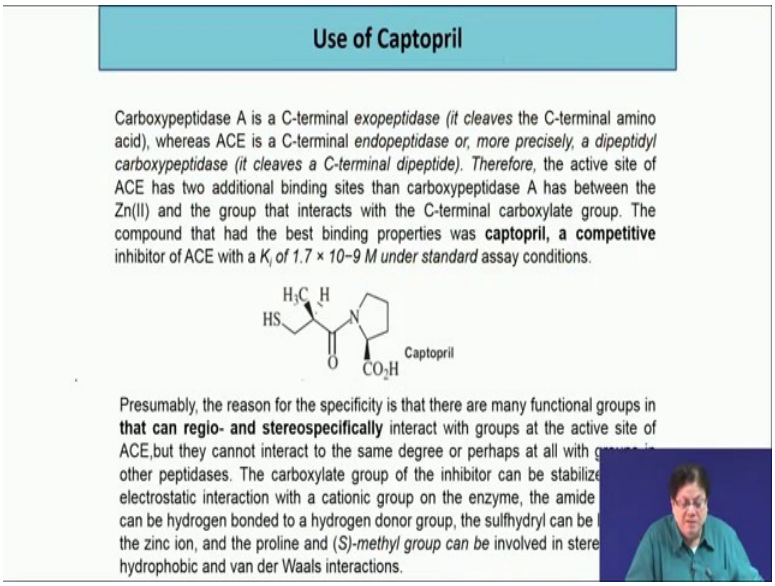

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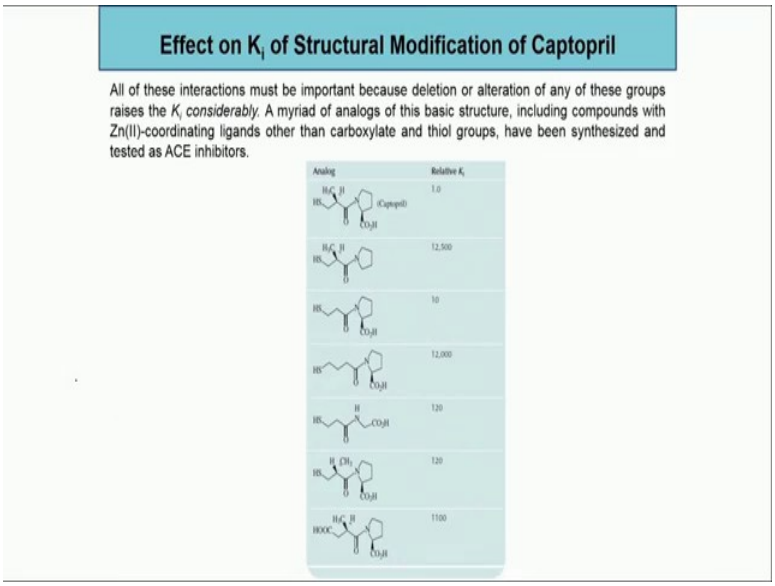

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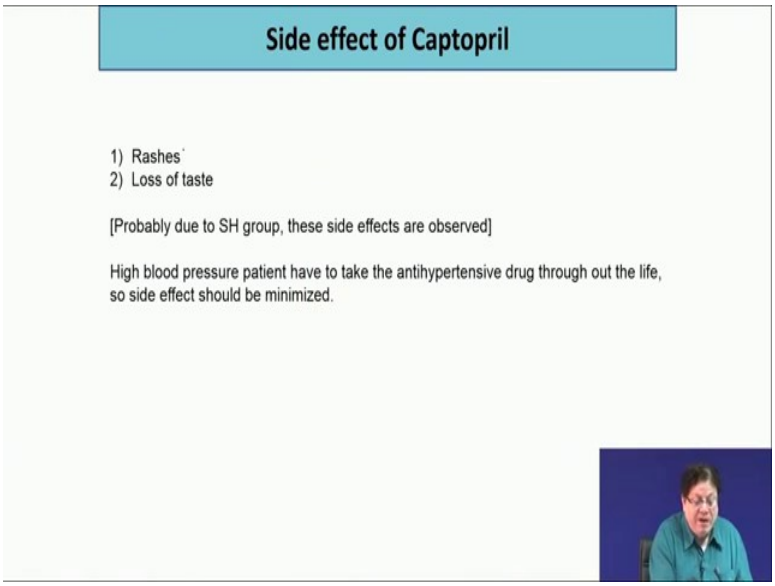

The captopril's effect was rashes that allergic reactions because sulphur causes allergy, like penicillin I told you and then loss of taste. So, people lost their appetite, loss of taste so, they are not eating much. So, this is probably due to SH groups. However, if you withdraw the drugs then they these are reversible effects; if you withdraw the drugs then again you get back your taste and the rashes also go out.

So, captopril is not the ideal drug; they realized that because this is one important issue that antihypertensive drugs is not that like antibiotics that I take a course of 7 days and then withdraw, but here you have to take almost lifelong. So, if you have loss of taste lifelong so, it is a miserable state of life. So, captopril is not the ideal drug.

So, then they started removing the sulphur and then see whether a better design can be can be done. Remember, in captopril, there is a methyl here, beta methyl then CH_2SH. Now, they were again back to the carboxy because carboxy derivative is the one like that benzyl succinic acid which was the inhibitor of carboxypeptidase A. So, they again went back to the carboxy because carboxy also creates chelation with the zinc.

So, they started doing the structure activity relationship; they tried different number of carbon atoms; then put this NH and CH_2, peptidomimetic chemistry and then after all these, you see there is a decrease in the IC_{50} value.

I write this compound. You have a proline that means, this CO_2 minus of course, proline is obligatory because you have to have this electrostatic interaction. Then they did not change the alanine methyl NH, then alanine is not connected by a peptide bond, but a CH because you cannot have a peptide bond because that will be then hydrolysed by the angiotensin converting enzyme. So, CH and then actually initially they have this compound CH_2CO_2H.

This CO_2H you can write in this fashion as a chelating group. So, you write CO_2 minus like this and this is the zinc. So, this is where the zinc is chelated. There is a pocket here methyl, there is a pocket here for the carboxyl. So, that is how it binds.

This has got an IC_{50} value of 2.4, but that is not very good. IC_{50} value should be less than 1 0.00 something or 0.0 something.

IC_{50} value will ultimately determine what is the dose of the compound you have to take. So, higher IC_{50} means; higher dose of compound you have to take higher dose means you have you run the risk of crossing the therapeutic index. So, lots of issues are there.

So, you have to lower it further. So, then people realized that in the carboxypeptidase or if you look at the peptides again, let us go back to the snake venom peptides; I think that will give you a very good idea. Accordingly, you have utilized the proline in your design you have utilized the alanine also for your design; the third one is on this side is what is called an aromatic amino acid.

So, what you have done? You have exploited the binding of the carboxy group of the proline; you have exploited the binding of the methyl and this carbonyl also has some hydrogen bonding interactions; you have exploited the binding of the zinc by utilizing a carboxyl here. But, what you have not done is exploitation of this binding hydrophobic pocket that is not yet done. So, in order to get a better inhibitor now, what you have to do? You have to additionally put this aromatic ring, so that it goes and binds to the pocket.

Now, here one nomenclature system I should tell you that if some protease hydrolyses this peptide bond, then with respect to that hydrolysable amide bond, the first amino acids on the N-terminus side will constitute the S_1 pocket; then if you go on to the N-terminus side further away from the actual peptide bond which is hydrolysed so, then

you have an S_2 pocket. Then you have on this other side it is a S_1' pocket, then S_2' pocket.

So, if I say that this is the angiotensin converting enzyme. Then in S_1 pocket there should be an aromatic amino acid.. It is a dicarboxypeptidase. So, the hydrolysis will be between the tryptophan and the alanine.

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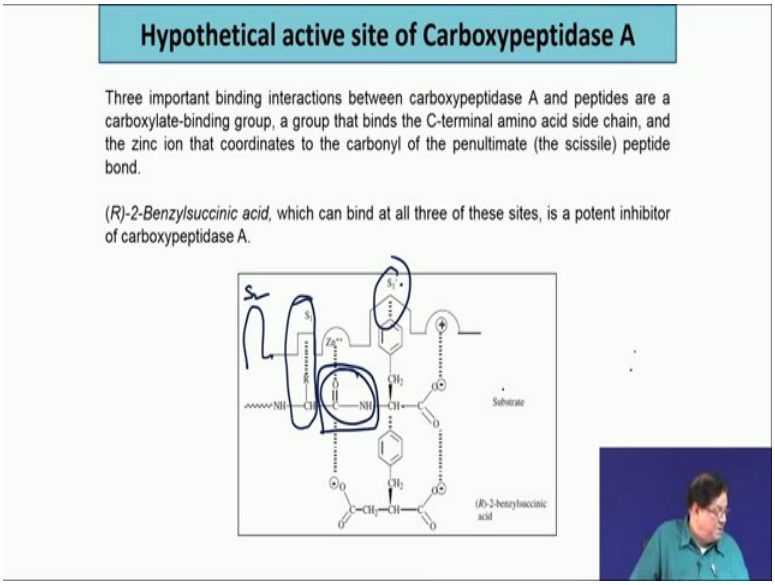

See here the peptide bond that is hydrolysed is this one, this is called the S_1 pocket. In the S_1 pocket you have phenylalanine or tyrosine, basically the aromatic amino acid. So, if there is another one here that will be S_2 pocket. So, the normal numbering goes to the N-terminus side and on the C-terminus side, you have S_1' pocket and S_1' pocket. So, proline is at the S_1'pocket. This is all in relation to the peptide bond that is being hydrolysed. So, you have not utilized that S_1 pocket. See the reference is the peptide. So, on the right side S_1', then S' second amino acid, third amino acid is S_3' and on the left side the first amino acid is S_1 the second one is S_2.

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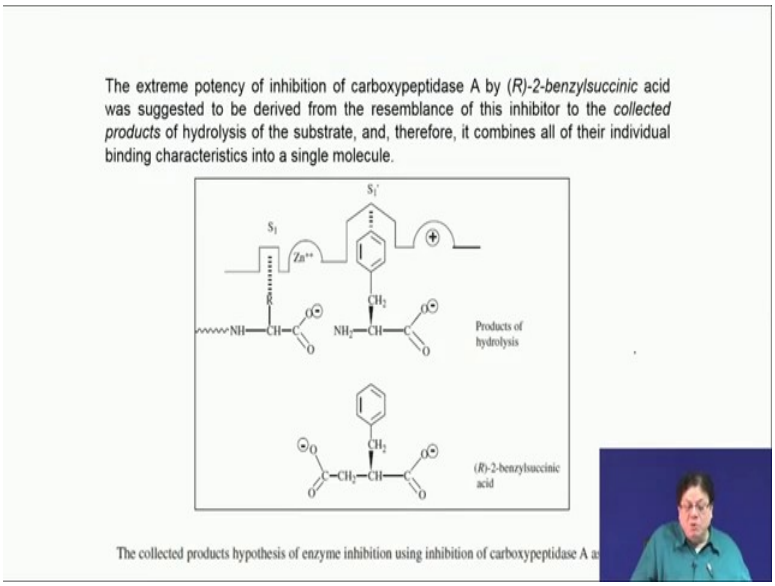

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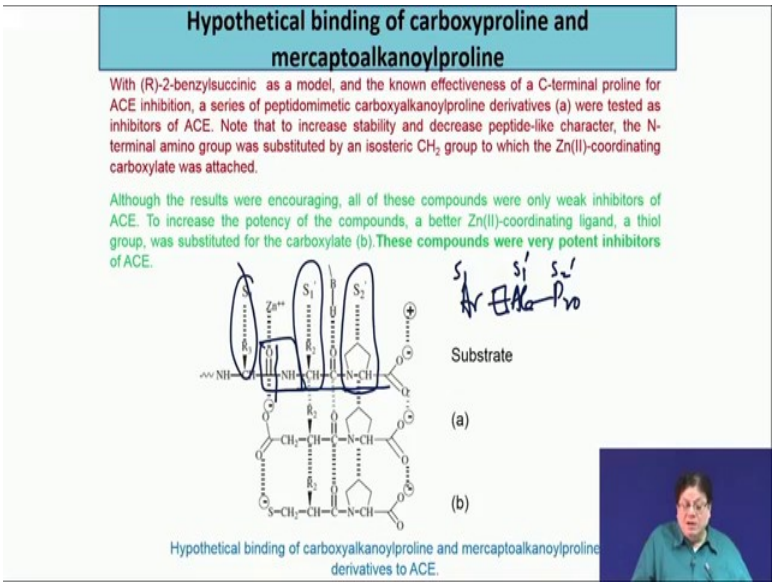

So, in this case it is a dicarboxy peptidase; that means, this dipeptide (Ala-Pro) goes off. You have a proline, you have alanine you have an aromatic amino acid. So, the bond that is being hydrolysed is between the aromatic amino acid and alanine.

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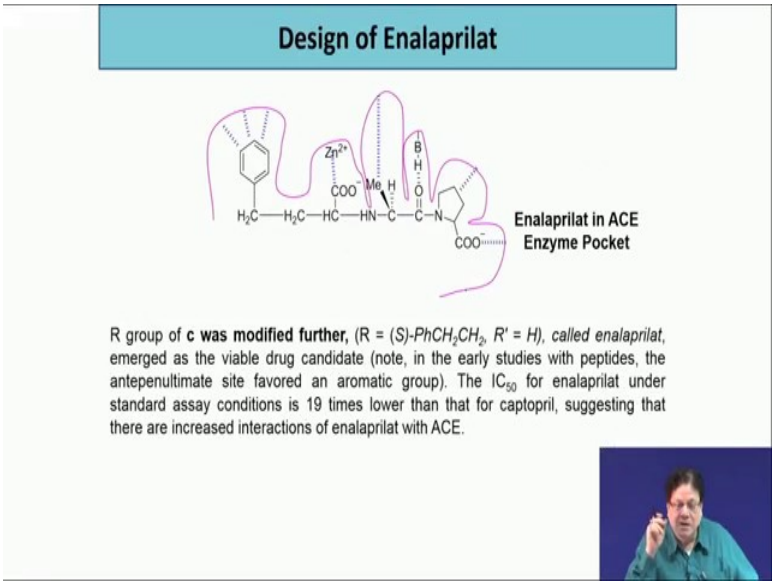

So, the scientist had not utilized the S_1 pocket yet. So, that had to be utilized. So, what they did now in order to develop this enalapril, or enalaprilat, they started working out. They have a proline, they have alanine, they have this CH and then on the CH they have this CO_2 minus which is a chelation to the zinc. And, now you know that here there is a pocket for the aromatic compound.

So, now, they put a CH_2CH_2Ph because that they have to monitor that how many carbons are required to get into the S_1 pocket, two CH_2s and then there is a Ph. So, now, there is a Ph here. So, they must have actually monitored; this is a structure activity first they attach direct Ph, then they attach CH to Ph, then they attach CH_2CH_2Ph and then found that this is the compound which they ultimately got.

This is what is called enalaprilat which has got an IC_{50} value is 0.0012. So, this ultimately became the drug. However, there is still some twist left in the discovery process what they found, once you have an inhibitor you have to do the PK PD studies. PK is basically ADME studies, absorption distribution, then metabolism and excretion. This drug unfortunately is quite polar and it was not absorbed properly from the gastro intestinal tract.

So, you are not getting the correct concentration of this enalaprilat in the blood stream; that means, it was not working as a as it was intended. That means *in vivo* activity was much less; that means, it was not absorbed; that means, it has got very poor absorption

property because it is quite polar. So, what will be the answer? You make it less polar. So, what they did, this carboxylic acid which was chelating to the zinc, they made it as an ester. If you block the carboxy as the ester, it loses it is potency as it cannot chelate to the zinc anymore; as long as it becomes carboxylic acid then it can chelate to zinc.

However, when you put ester it has become more lipophilic now; I told you about the logP value. So, it satisfies the logP value, if you put the ester. So, now, it is absorbed from the gastro intestinal. It has got good absorption property and the beauty is that as soon as it goes into the blood stream, there are many non-specific esterases in the body which hydrolyses it and forms the enalaprilat in the blood stream. So, basically this is what is called a prodrug. This is not the drug, this is a prodrug, this is has got very good pharmacokinetic properties.

So, it goes, gets absorbed and distributes and the non-specific esterases present in the blood hydrolyse it and converts it into this enalaprilat and now enalaprilat acts as a very good antihypertensive agent. So, now, there are new generations of antihypertensive agents; Ramipril is a drug which is also used as an antihypertensive agent. However, enalapril has been prescribed to many patients these days in order to manage the hypertension.

Only before I stop, I also I touched the point a little bit that you can also target the angiotensin 2 receptors because angiotensin 2 exerts its effect by binding to its receptors. So, if you can use antagonist of this receptors that will also act as the antihypertensive agent. And, in fact, there are drugs which are called angiotensin 2 receptor antagonist like telmisartan, olmesartan, basically they go and bind and block the angiotensin 2 receptors.

So, there are two types of drugs based on this angiotensin renin system. One is the ACE inhibitors and another is the angiotensin 2 receptor antagonist; both are in the market now and that is the major blockbuster drugs available in the market for management of hypertension.

Thank you.