Medical Chemistry
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Endogenous Compounds, Peptidomimetics and Oligonucleotides as Drugs

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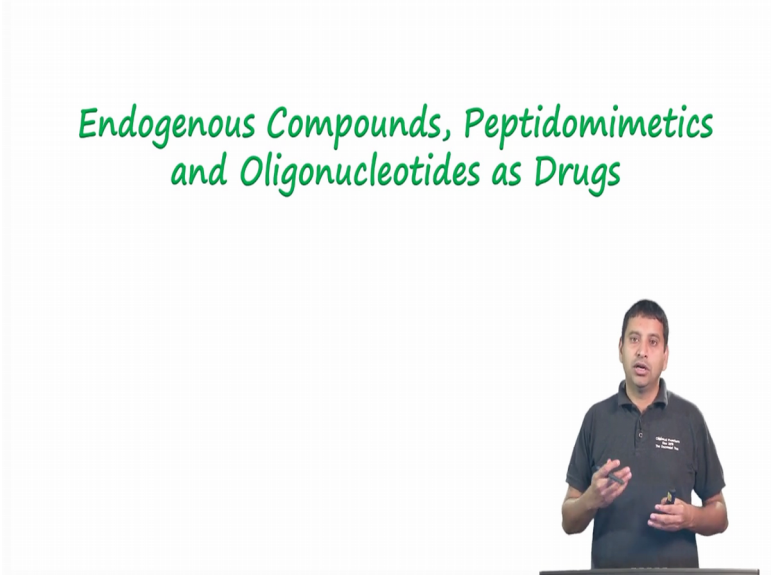

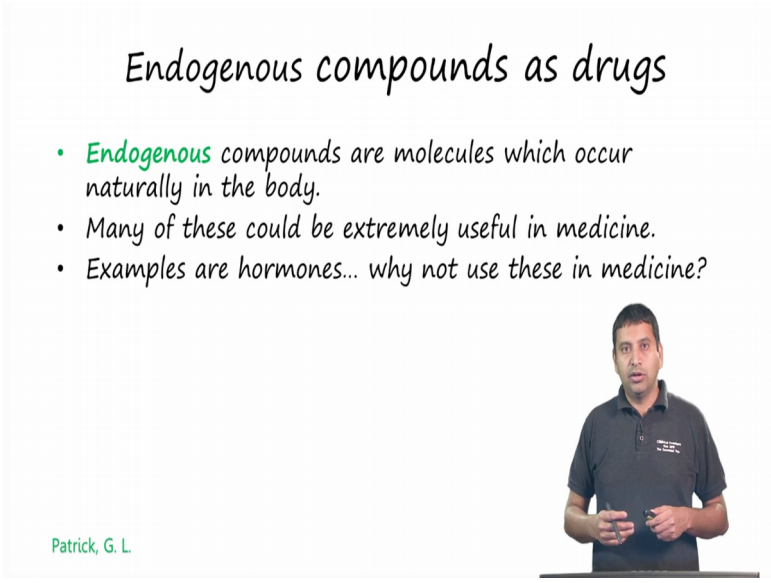

In today's lecture, we are going to look at the possibility of using Endogenous compounds, that is these are the compounds that are produced inside our body. And there is another class of compounds known as Peptidomimetics, we will look at the definition a little bit later. And as well as oligonucleotides as drugs. So, we have just been looking at various strategies for the design but we want to go back and look at some of the possibilities and examine them and

critically evaluate how good these strategies are. So, just to remind you, endogenous compounds are basically the molecules which occur naturally in the body, okay.

So hormones or neurotransmitters, these are examples of molecules which are present inside the body. And of course many of these could be extremely useful because you know the imbalance in the levels of these neurotransmitters or hormones sometimes leads to disease. So, the question is why not use these in medicines. Okay.

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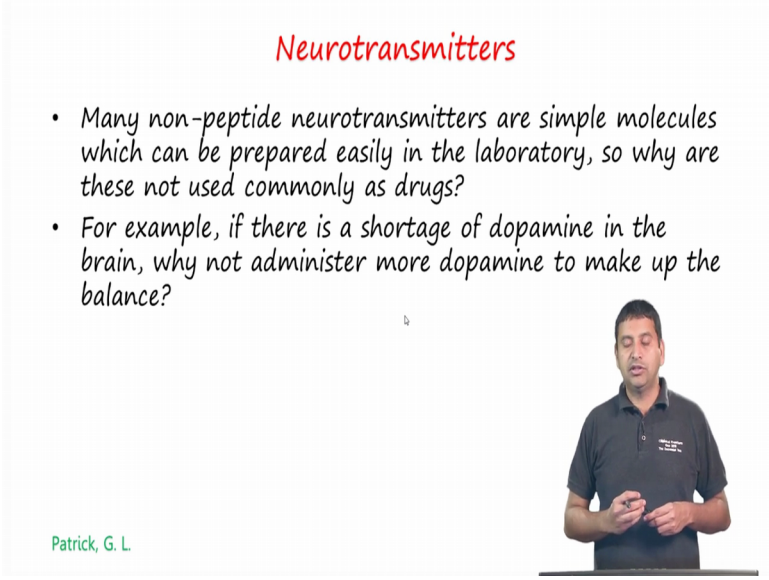

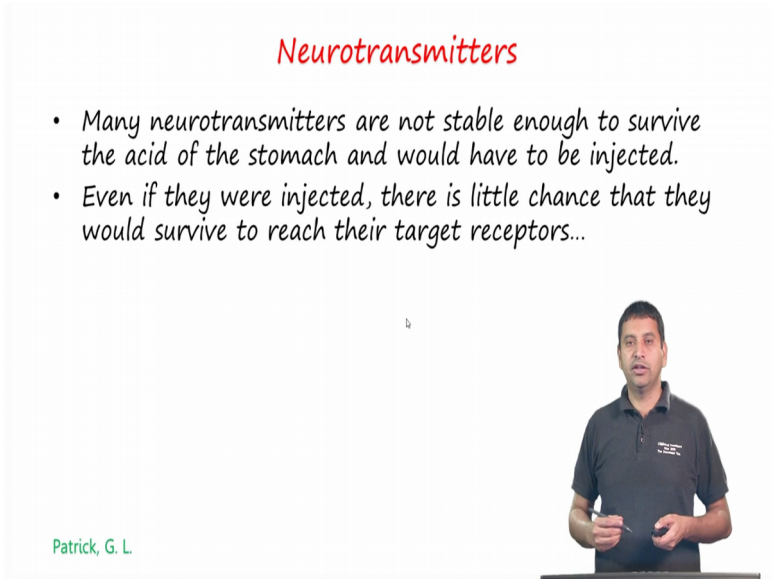

So let us start with the neurotransmitters. So there are a number of non-peptide neurotransmitters which are present in the body and they are very simple molecules and they can be prepared very easily in the lab. So the major question that one asks is why are these

not used very commonly as drugs? So, the classic example is dopamine, so we have already looked that this in great detail. The dopamine levels need to be elevated in certain kinds of diseases such as Parkinson's disease and so one could think of this administering dopamine, so that we can maintain the make-up for the balance.

So why is this not done? So the problem with neurotransmitters is that they are, they have not been designed to travel long distances within the body, they are typically used you know with very short range signalling and therefore not very stable, okay. So for example if we consume medicine or a pill containing this neurotransmitter, it is quite likely that they will get decomposed into stomach, because of the high acidity. And now, therefore you will probably have to inject it. So then injection of course is going to help us by pass the 1st pass effect, but the problem is that it may not survive until it reaches the target receptor.

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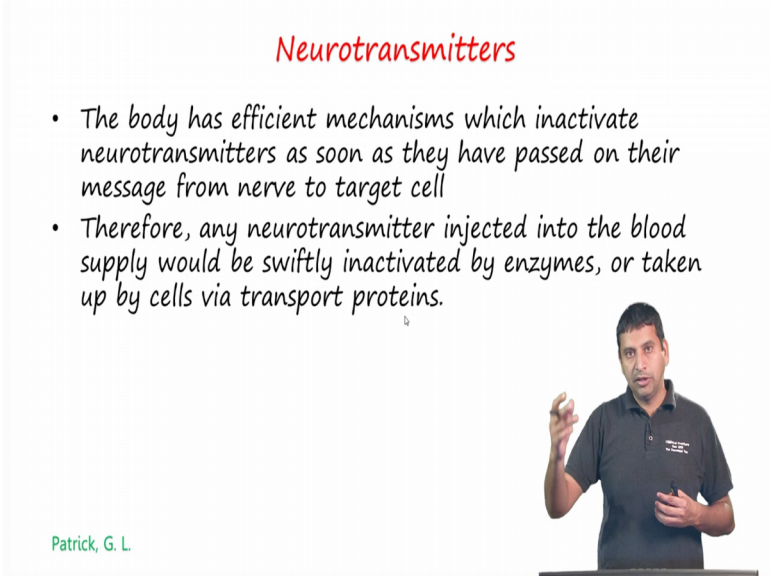

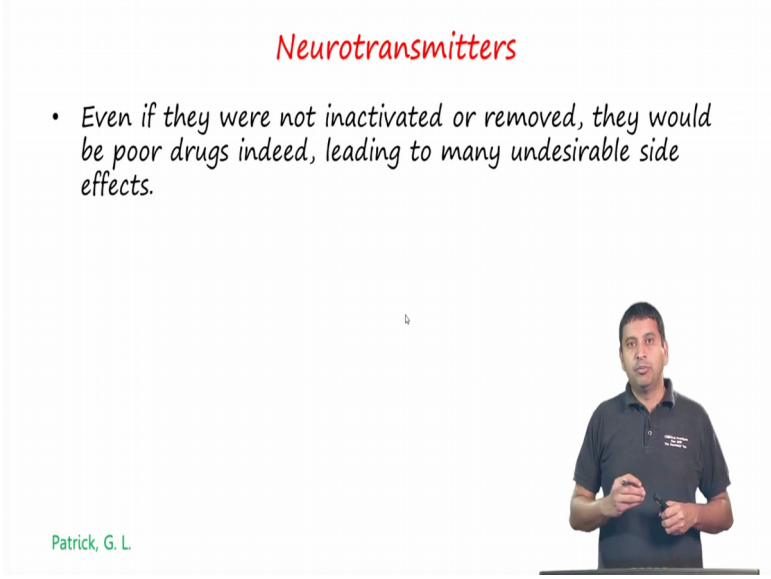

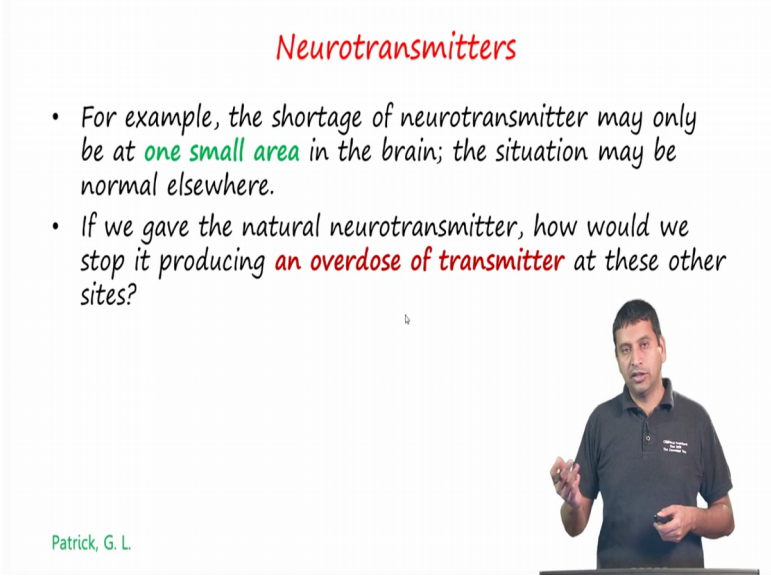

So the major problem with using neurotransmitters or the natural neurotransmitters is that they will not be stable enough and they may not survive to reach their targets receptors. And one of the reasons for this is because the body has efficient mechanisms which are used to inactivate these neurotransmitters. So for example, if the neurotransmitter is not inactivated, then they are going to continue to pass on the signal from the nerve to the target cell and that is not very useful.

So there are endogenous mechanisms natural mechanisms by which these neurotransmitters are going to be metabolised. Okay. Typically they are very short lived, so any neurotransmitter, that is injected into the blood supply is going to be inactivated by enzymes or what happens is that they are going to be taken up by self via transport proteins. So both of these are going to result in a loss of the amount of the neurotransmitter available to reach the

target side. So, even if they were not inactivated or removed , they would really be poor drugs because they would lead to many undesirable side-effects.

So for example if you want the drug to be active in the lungs, while it is going to be activated in all areas including the lungs. So, shortage of neurotransmitters maybe only in a small area in the brain, but in other areas in the brain, it might be quite normal or in other areas of the body, it would be quite normal. So, if we give the natural neurotransmitters, then it would be impossible for us an overdose of the transmitter and it will be able to trigger the signalling in other areas as well.

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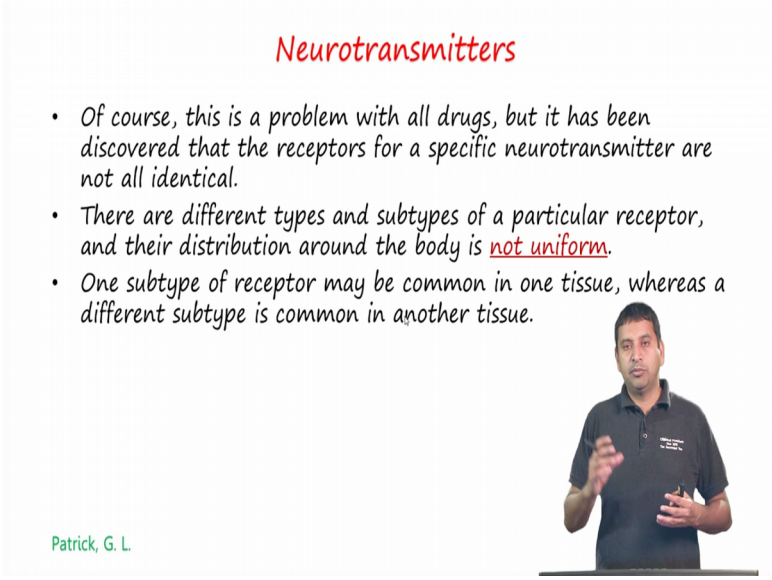

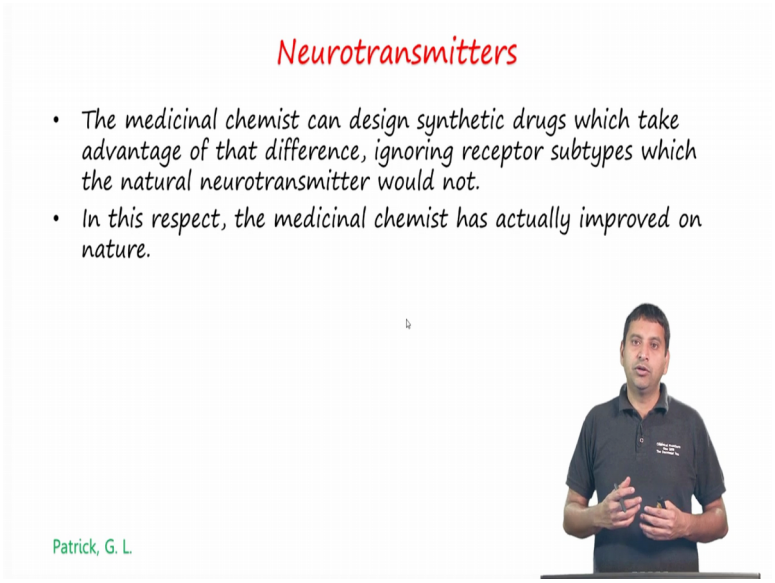

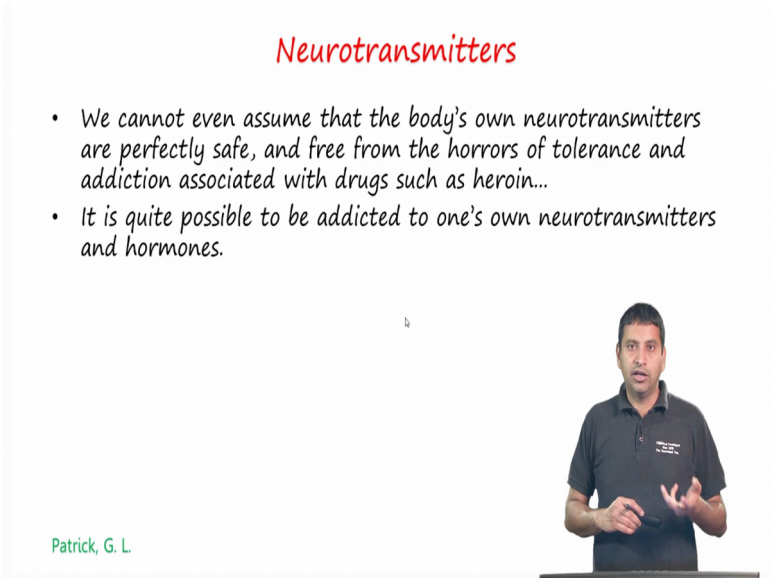

Of course this is a problem, not just with new transmitters but all drugs. It has been discovered that the receptors for a specific neurotransmitter are not identical. So, we have looked at this previously. So, therefore there are different types and subtypes of a particular receptor. And the distribution around the body is also not uniform. So, if we use the natural neurotransmitter, there is no chance for us to distinguish among these various subtypes. However by using a synthetic molecule such as a drug, it is possible that we may be able to distinguish these subtypes.

So once a type of a receptor may be common in one tissue, whereas another subtype is common in another tissue. So the medical Chemistry can now design an appropriate synthetic drug, which takes advantage of this difference and which will help us target a particular receptor subtype while the natural new transmitter would not be able to do it. Okay, so in this respect medicinal chemist actually has an opportunity to improve on nature. So the other part of it is that we cannot assume that the body's own neurotransmitters are perfectly safe.

So for example if one is being constantly injected with the body's neurotransmitter, then it is possible that there is tolerance and addiction associated with these products. So, classic example is heroin, heroin tolerance and addiction are quite commonly observed. So, it is possible that one can be addicted to one's own neurotransmitter and hormone.

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Neurotransmitters

- Some people are addicted to exercise and are compelled to exercise long hours each day in order to feel good.
- The very process of exercise leads to the release of hormones and neurotransmitters which can produce a 'high', and this drives susceptible people to exercise more and more.

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Neurotransmitters

- If they stop exercising, they suffer withdrawal symptoms, such as deep depression.
- The same phenomenon probably drives mountaineers into attempting feats which they know might well lead to their death.
- The thrill of danger produces hormones and neurotransmitters which, in turn, produce a 'high'.
- This may also explain why some individuals choose to become mercenaries and risk their lives travelling the globe in search of wars to fight..

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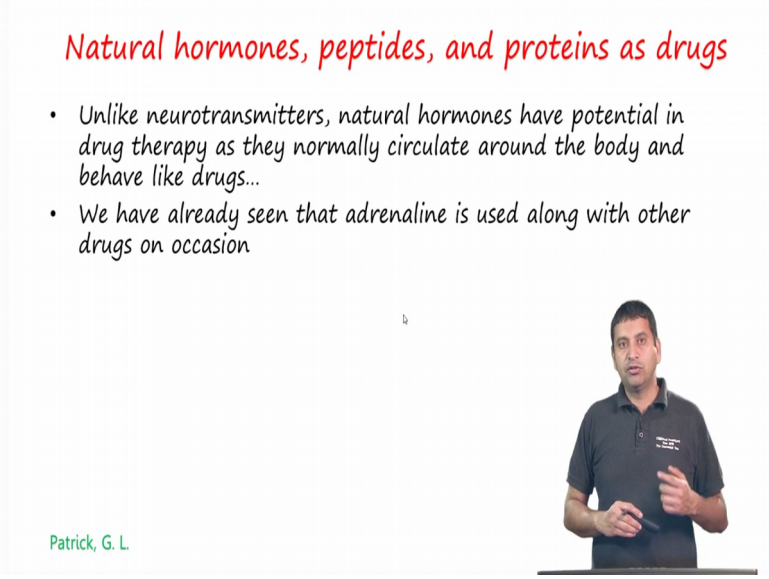

So a parallel example here is that some people are addicted to exercise, okay, and so and they are compelled to exercise for very long hours in each day in order to feel good. Because during the process of exercise, which I would highly recommend, that you know, it releases the hormones and neurotransmitters which can produce good feeling for what is known as a 'high'. And so there are certain types of people who are susceptible to, you go to this sort of feeling and the exercise more and more to achieve this high.

But as a general concept, it is a good idea to exercise regularly and need not be for several hours but it can at least be for 45 minutes to 1 hour. So, these people who are addicted to this exercising actually suffer what are known as withdrawal symptoms, which we have looked at previously, such as deep depression. And so this is the same phenomena in that drives risk-taking people, for example mountaineers to attempt feats such as climbing very tall mountains, which they are probably quite sure that it will lead their deaths, okay.

So that thrill seekers or a thrill of a danger produces these hormones and neurotransmitters which can again in turn produce a high. It also explains why some individuals choose to become mercenaries and risk their lives by travelling around the globe in search of wars to fight. So, the natural neurotransmitter as the drug may not be the best concept to take forward. So, unlike neurotransmitters, hormones have the potential in drug therapy because they normally circulate around the body and behave pretty much like drugs.

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Natural hormones, peptides, and proteins as drugs

- Most hormones are peptides and proteins, and some naturally occurring peptide and protein hormones are already used in medicine... such as **insulin** , **calcitonin** , **erythropoietin, human growth factor**, **interferons**, and **colony stimulating factors**...

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Natural hormones, peptides, and proteins as drugs

- Isolating and purifying a hormone from blood samples is impractical because of the tiny quantities of hormone present.
- It is far more practical to use **recombinant DNA techniques**, whereby the human genes for the protein are cloned and then incorporated into the DNA of fast-growing bacterial, yeast, or mammalian cells.
- These cells then produce sufficient quantities of the prot

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Natural hormones, peptides, and proteins as drugs

- Many endogenous peptides and proteins have proved ineffective though.
- This is because peptides and proteins are highly susceptible to digestive and metabolic enzymes, poor absorption and rapid clearance from the body...

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So, we have already seen for example you know, when there is a sedative that is given when there is an anaesthetic that is given intramuscularly, Adrenaline is injected along with it so that it takes time for the sedative to release, to be distributed across the body. This is for the local anaesthetic. So, most hormones are peptides and proteins and some naturally occurring peptide and an protein hormones are already used in medicines. For example, insulin is already been very commonly used in diabetes, calcitonin and Arithropoitin and so on, these are already proteins which are used quite commonly.

The other problem with using a hormone is in purifying. So, isolating and purifying a hormone from blood sample is quite impractical because there are very really tiny quantities of the hormones present. So, what is used is a technology known as recombinant DNA technology, which we were already looked at previously. So here are human genes for the protein are cloned and then incorporated into the DNA of fast-growing organisms such as bacteria, for example E. coli or yeast and in some cases mammalian cells.

And then these cells then overproduce these hormones and then we isolate the sufficient quantities of the protein by running this time and again. So, however many endogenous peptides and proteins have proved quite ineffective, so the reason for this is that they are highly susceptible to digestive and metabolic enzymes. So, there are enzymes which are present in our, as they will be looked at proteases for example, which can cleave these peptides and proteins. Not just that, they are also very poorly absorbed because of their highly polar nature, they are quite poorly absorbed and because of the high polarity, they also rapidly cleared from the body.

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Natural hormones, peptides, and proteins as drugs

- Proteins can also induce an immune response result in severe side-effects

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Natural hormones, peptides, and proteins as drugs

- Solutions to some of these problems are appearing, though.
- It has been found that linking the polymer **polyethylene glycol** (PEG) to a protein can increase the latter's solubility and stability, as well as decreasing the likelihood of an immune response

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Natural hormones, peptides, and proteins as drugs

- PEGylation, as it is called, also prevents the removal of small proteins from the blood supply by the kidneys or the reticuloendothelial system.
- The **increased size** of the PEGylated protein means that it is not filtered into the kidney nephrons and remains in the blood supply.

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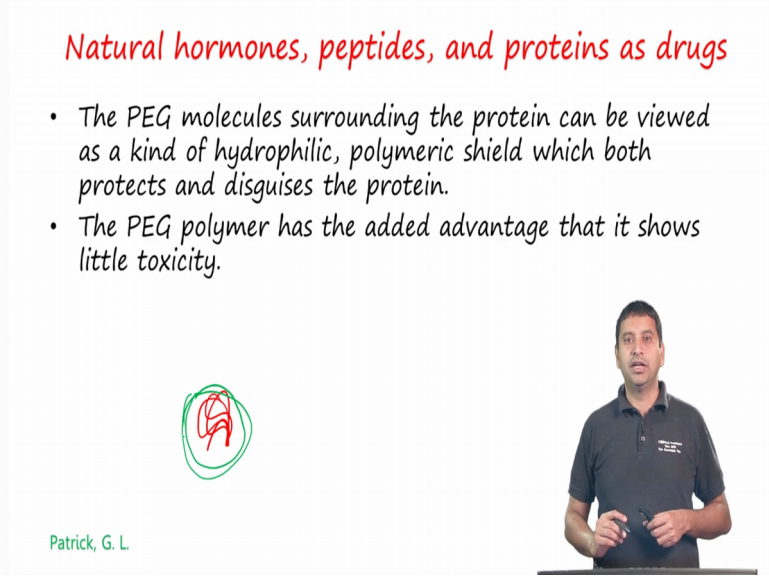

So together all of these concepts are important in designing endogenous peptides or proteins as a drug. So, also an important concept with protein for example, these are large macromolecules, is that they can induce an immune response, okay. So, since we are using recombinant DNA technology, there may be elements of nonhuman, you know, character to the macromolecules which can elicit an immune response. And these can cause pretty severe side-effects. So, one of the ways in which we can solve this problem is that you know, you can incorporate water-soluble polyethylene glycol on the polymer. So what this does is that once you have a protein, you can decorate it all covalently modify it with a PEG chain, which is called a polyethylene glycol chain.

So polyethylene glycol is nothing but your and so on, right. So this is a PEG chain, so you can take this and incorporate it into a protein. So, what it does is that of course it increases the solubility and also because it forms a layer around the protein, it increases the stability as well. Now because the polyethylene glycol is like homogenous putting around the molecule, it decreases the likelihood of an immune response. PEGylation as it is called, also prevents the removal of small proteins from the blood supply by kidneys or the endothelial system.

This is probably because of the large size of the PEGalyted protein. So, during the filtration process in the kidney, because these PEGalyted proteins are quite large, they are not filtered into the kidney enough and remain in the blood supply. So, together PEGylation is one of the important strategies to deliver protein. The PEG molecules surrounding the protein can be viewed as a kind of hydrophilic polymeric shield which both protects as well as disguises the protein.

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Natural hormones, peptides, and proteins as drugs

- The enzyme **adenosine deaminase** have been treated in this way to give protein-PEG conjugates for the treatment of **severe combined immunodeficiency** (SCID) syndrome, which is an immunological defect associated with a lack of **adenosine deaminase**

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Natural hormones, peptides, and proteins as drugs

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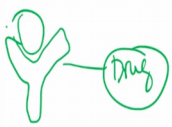
Antibodies as drugs

- Antibodies are used to recognize chemical signatures of particular cells or macromolecules...
- They are used to carry drugs to specific targets
- Several antibodies are used as drugs now...



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So let us say you have a protein here and around this you have a sort of a shield of this polyethylene glycol layer. So, it also has an added advantage, because polyethylene glycol polymers are extremely well tolerated by the body and they do not show any toxicity. So, the enzyme, adenosine deaminase has been used you know in a similar manner and protein PEG conjugate has been prepared. And this conjugate has been used for the treatment of severe combined amino deficiency, known as SCID, okay.

So this syndrome is associated with an immunological defect, which is because of lack of adenosine deaminase. So, therefore you can deliver this and I am adenosine deaminase has a PEGalyted form in order to sort of increase the levels of these enzymes in the body. These conjugates are these PEGalyted conjugates have much longer plasma half-life than the preferred protein alone and they are less likely to produce an immune response.

The next endogenous molecule is an antibody and antibodies are, we have already looked at previously are quite commonly used as drugs now and a lot of biotechnology companies are now producing a large number of antibodies or antibody-based drugs with the aid of genetic engineering and monoclonal antibody technology. So, antibodies are used to recognise chemical signatures of particular cells or macromolecules.

There also used to carry drugs to specific targets. So, here for example we were already looked that, the antibody is conjugated to a drug and this is used for targeting a particular site and the drug is delivered along with it. There are a number of antibodies which are used as drugs.

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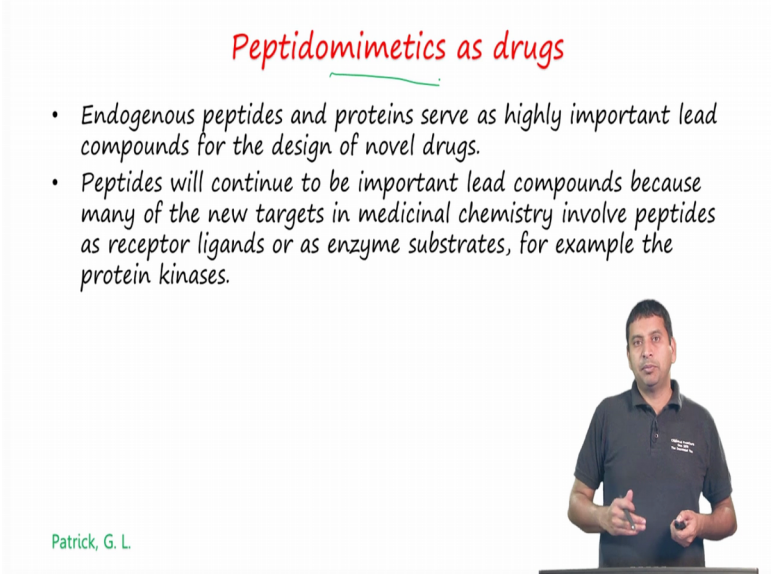

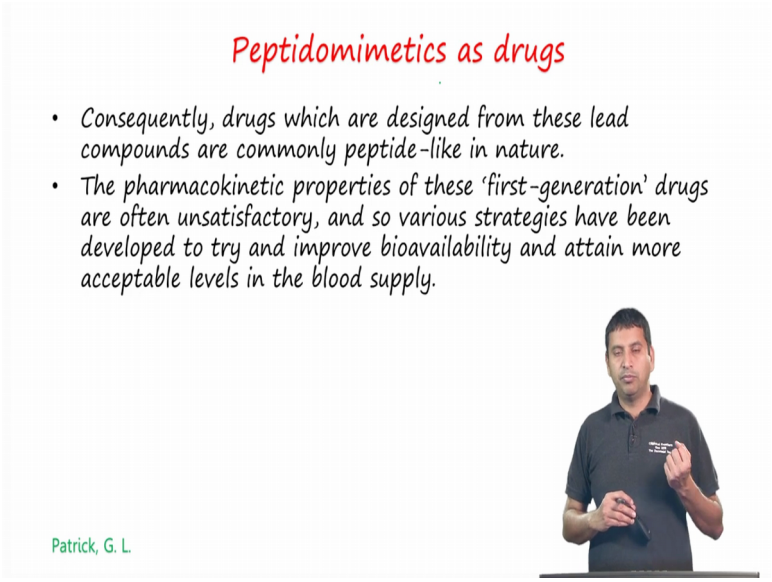

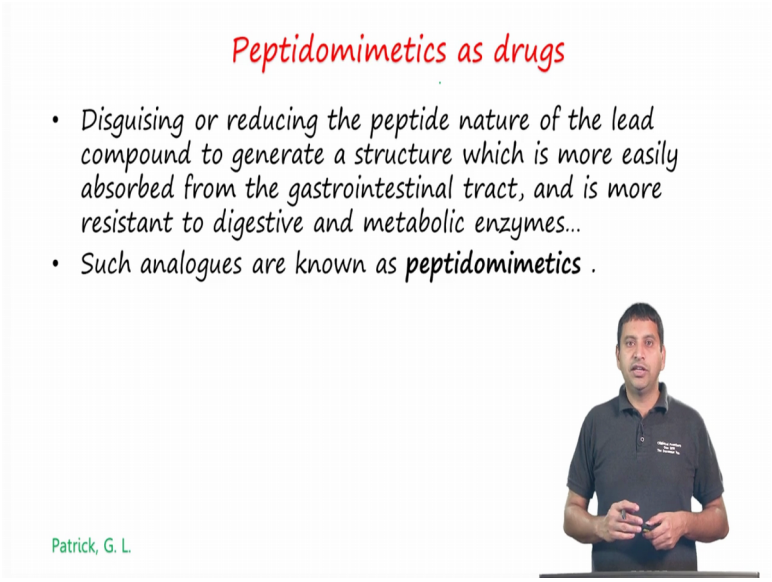

The next major molecule to consider are of course endogenous peptides. But we have already looked at some of the pros and cons of using this endogenous peptides and proteins. So one can sort of work around this by using what are known as Peptidomimetics. Okay, so mimetic means to mimic or to look like, okay. So this Peptidomimetics are molecules which are look like or feel like or behave like peptides. Okay. So because peptides continue to be important lead compounds, a lot of medicinal chemistry has worked around peptides and because peptides as receptors ligands or enzyme substrates are very commonly found, okay.

Therefore we do not want to give up on trades altogether, so therefore we would want to be able to deliver a peptide or a peptide like molecule which is known at Peptidomimetics. So, consequently drugs which are designed from these lead compounds which are peptide like in nature, have to be taken forward. So, the pharmacokinetic properties of these first-generation

drugs of using peptides are many times unsatisfactory, okay. And so a number of strategies have evolved to improve the bioavailability and to achieve a higher level or acceptable level of these molecules in the blood supply.

So therefore disguising or reducing the peptide nature of the lead compound to generate a structure which is more easily absorbed from the GI tract and more resistant to Digestive and metabolic enzymes is highly desirable. And these molecules which are known as Peptidomimetics.

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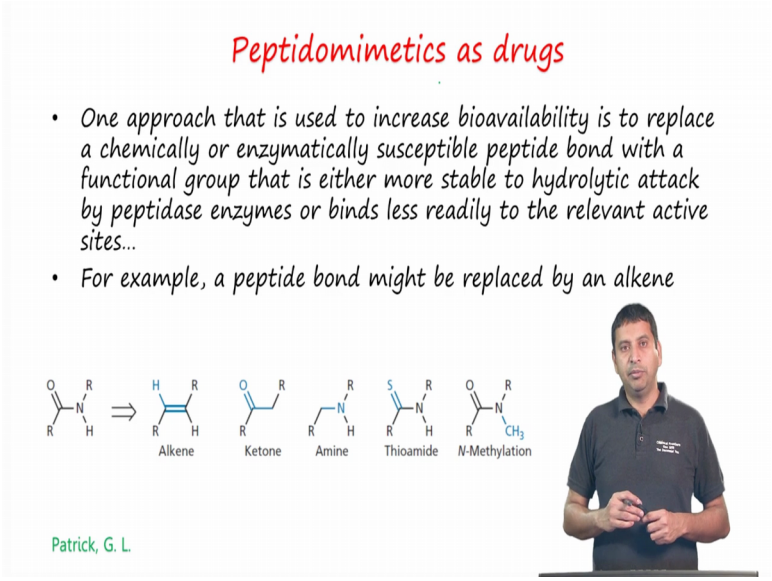

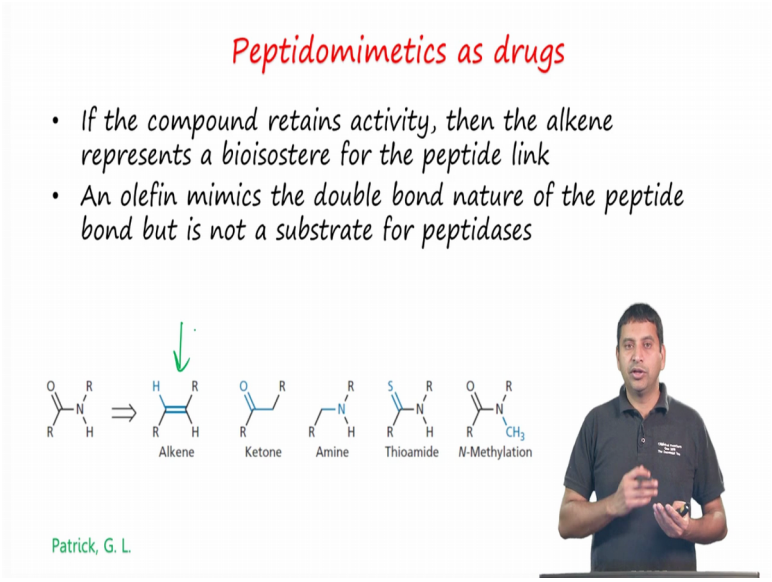

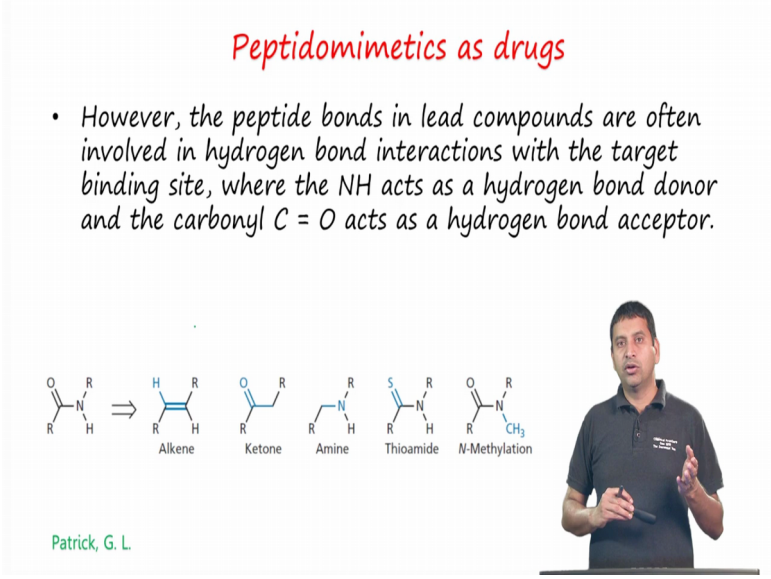

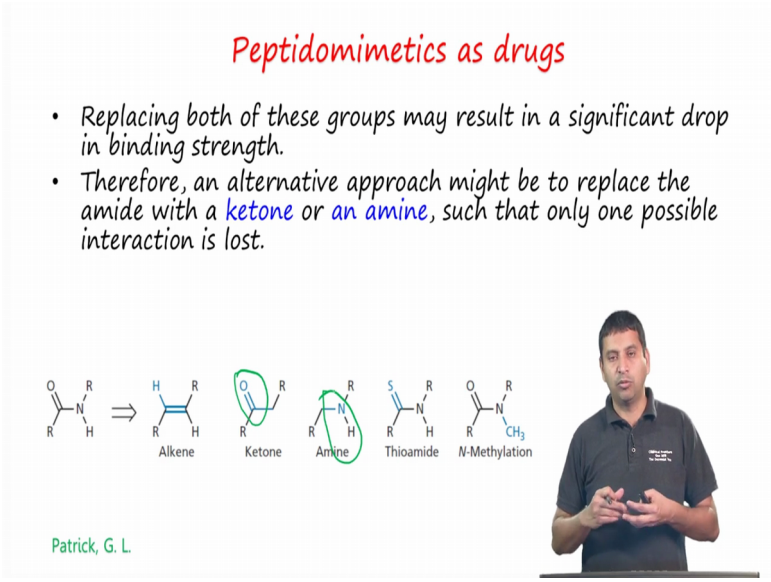

So, one approach that is used to increase the bioavailability is to replace a chemically or enzymatically susceptible peptide bond. Okay. So here is a normal amide or peptide and so these are enzymatically susceptible. Right. And in order to prevent this, one could use a range of these functional groups. The 1st example we will take is to look at a peptide bond which is replaced by an alkene. Okay. So here if you replace the peptide bond with an alkene, then this alkene is a bioisostere for the peptide linkage.

The olefin mimics the double bond nature of the peptide bond, so because we have already looked at previously that the peptide bonds have significant double bond character, okay. But clearly the lesson or the alkene is not a substrate for proteases, right or peptidases. And therefore this is extremely stable during the, during metabolism, especially to peptidases. However the peptide bonds in many lead compounds are often involved in hydrogen bonding

interactions. So you have, you know hydrogen bonding interaction possibly due to the carbon and as well as amine.

And now once we remove this amine altogether, and replace it with the knowledge and, then you are removing the hydrogen bonding donor and accepting capability and therefore you may lose activity. Okay. So this possibly results in a significant drop in the binding strength, right. The alternative is to replace this amide with a ketone because the ketone still has the carbonyl group on it. Or you can replace it with an amine, right. So here only one of the 2 interactions is being lost and therefore it might help compensate for the loss.

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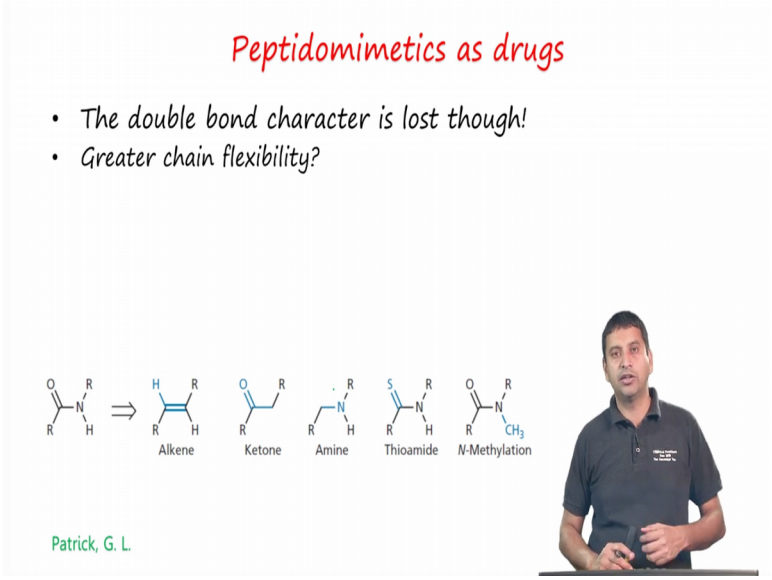

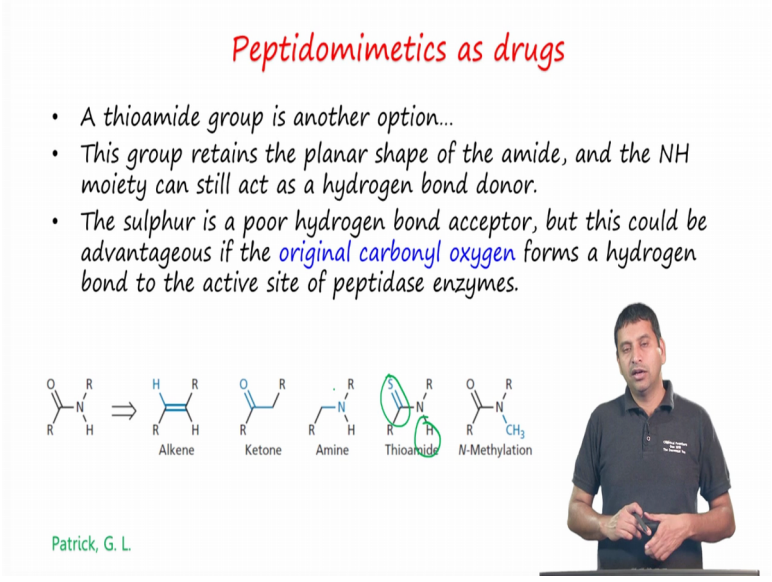

However the problem with these 2 approaches is that here the double bond character is completely gone. So there is greater changeless civility also because the amide bond is more

restricted and therefore you do not have the same sort of flexibility. The next option, that is typically considered is a thioamide. So, here instead of oxygen, you have a sulphur, okay. So, this group retains the leadership of the amide and the NH Moiety can still act as a hydrogen bond donor.

However the sulphur bond being quite large and quite less in electrical negativity compared to the oxygen is a poor hydrogen bond acceptor. So, this strategy would be useful if the original carbonyl oxygen forms the hydrogen bond to the active side of peptidase enzymes. So if a peptidase enzymes bind to the carbonyl took leave it, now by putting in a carbon double bond sulphur, you are reducing the possibility of it getting hydrolysed by peptidase enzymes.

Another approach is to retain amide as it is it is but to protect it or to disguise it. So, one simple strategy that can be used is to just methylate the nitrogen of the amide group. So, this keeps pretty much the same properties but it improves the stability of the molecule towards proteases.

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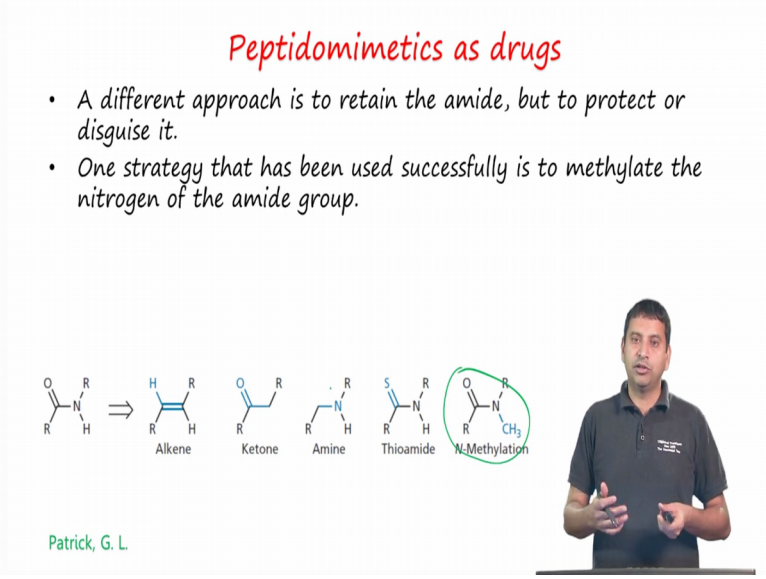

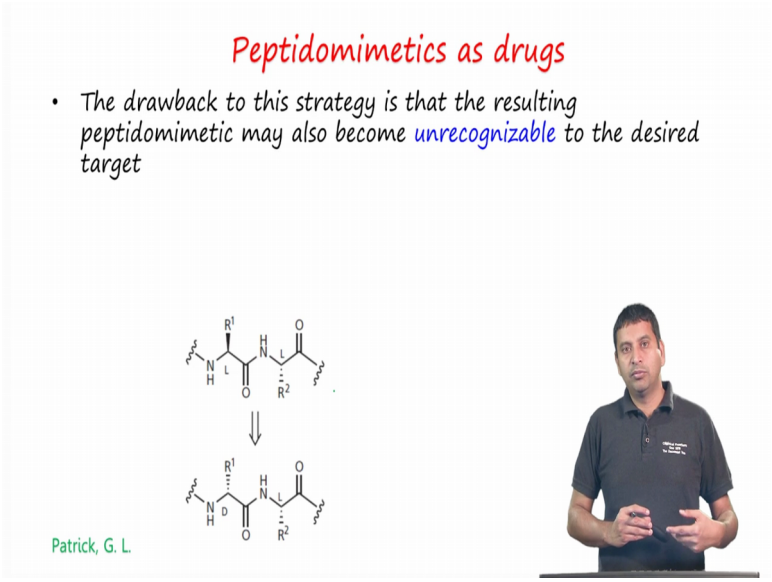

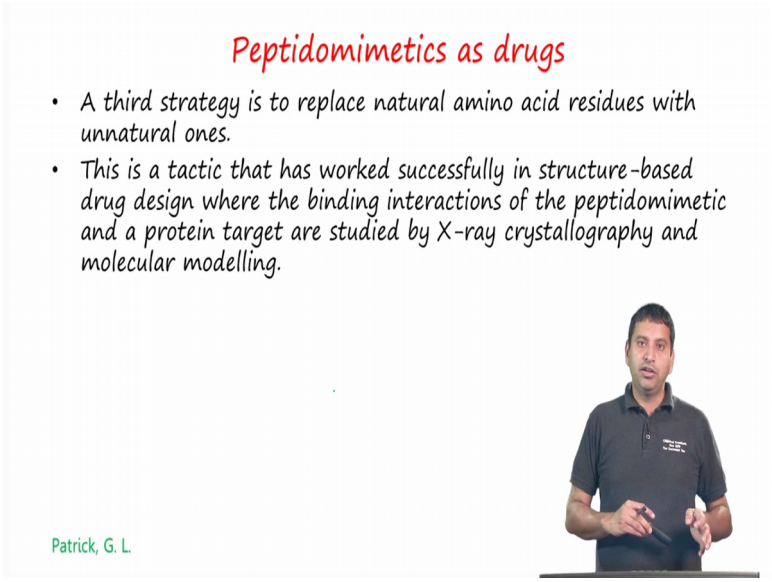

So the methyl group acts as a steric shield and prevents the important hydrogen bonding interaction that is supposed to take place between the NH of the original amide and the active site of peptidase enzyme. So therefore, not that this steric shield is present, the rates of hydrolysis significantly go down and so N methylation is also a one way to generate amide like or to retain the properties of the amide as a drug. Another unrelated or a completely different strategy is to flip the enantiomer that we use.

So for example if we replace the L enantiomer that are normally present in proteins with the D enantiomers, then what happens is that this makes the relative orientation unrecognisable to digestive or metabolic enzymes. Especially if that site change is involved in binding interactions. So here for example, you have an L amino acid and if you convert, you have 2 L

amino acids but if you convert one of these two to D amino acid, then it is possible that this may not get hydrolysed as well as original molecule.

But the problem is that by making this change, we also might make the the peptide unrecognisable at the desired target. We will be looked at previously that the relative positions of these site chains may become important in binding 2 targets. A 3rd strategy is to replace the natural amino acid residue with an unnatural amino acid, okay. So here the unnatural amino acid, says this is not present naturally, there are situations where these are going to survive much longer than the normal or the natural ones.

So this is a tactic that has worked successfully in structure based drug design. So once we have a map of the binding interactions of the let us say the active site with the ligand, so these are the major binding interactions. Now this is again obtained by x-ray crystallography and molecular modelling. So once we have these interactions setup, then we can design a new Peptidomimetics compound which can have the same or a similar interactions but do not have the natural amino acid as the component of the molecule.

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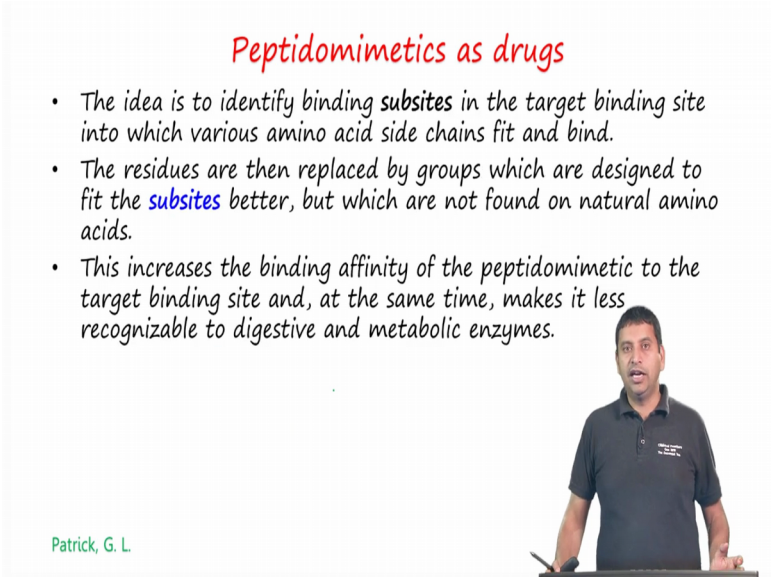

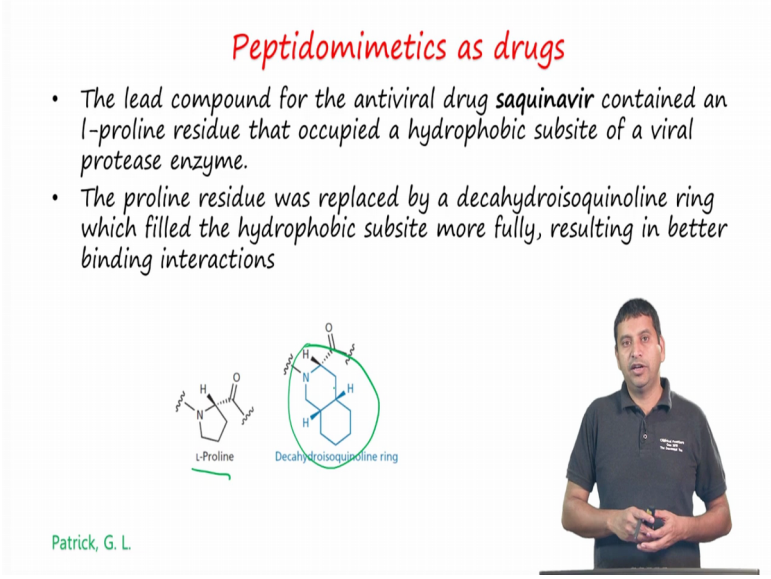

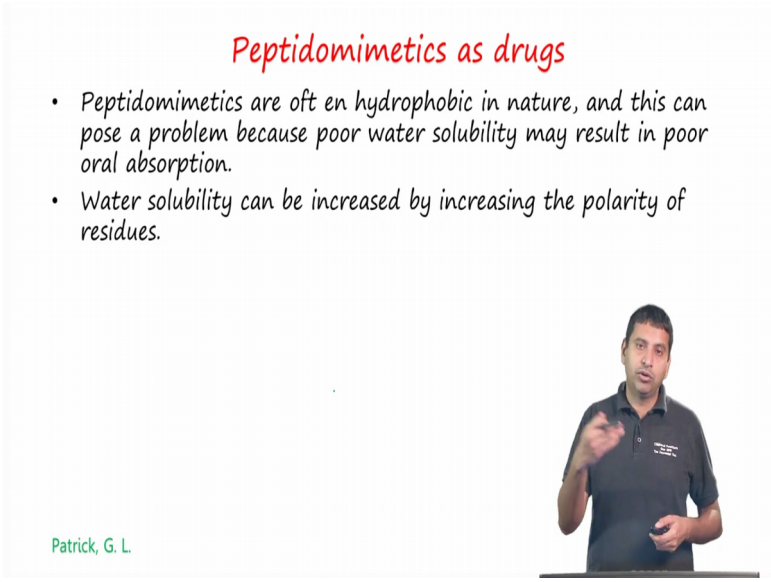

Once we identify these binding subsites in the targeting binding site and then what we do is we start designing molecules which can fit these subsites much better. Okay, but the major condition here is that we do not use the natural amino acids and we use what is known as the unnatural amino acid. So what this could lead to is actually it could lead to an improved binding affinity of the Peptidomimetics because now we are adding in more components of binding to the molecule.

At the same time it is clearly less recognisable to the digestive and metabolic enzymes. So the lead compounds for the anti-viral drug saquinavir contained and L proline residue can occupy the hydrophobic side of viral protease enzymes. So, here is the L proline residue and it occupied a hydrophobic site. So during the design, drug development, this proline residue

was actually replaced by a decahydroisoquinoline ring. Okay, so not only was this little bit more resistant to hydrolysis, but it also helped in fitting it in the binding site.

So one of the problems with Peptidomimetics is that they are often quite hydrophobic in nature because you are now going to add more carbons for example. And this can pose a problem because they are very poorly water-soluble, right. So when these molecules are to be taken orally, there are quite poorly absorbed. So of course what we can do is we can improve the water solubility by increasing the polarity of residues. We have already looked at these strategies in our previous lectures.

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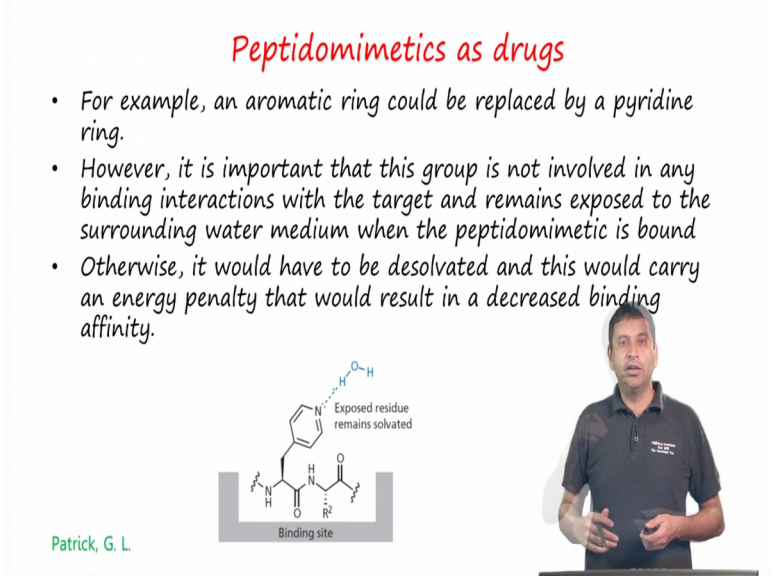

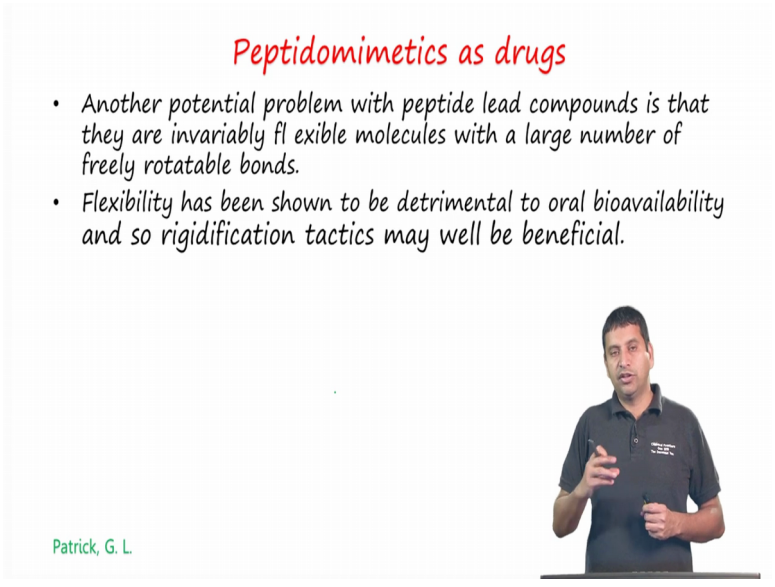

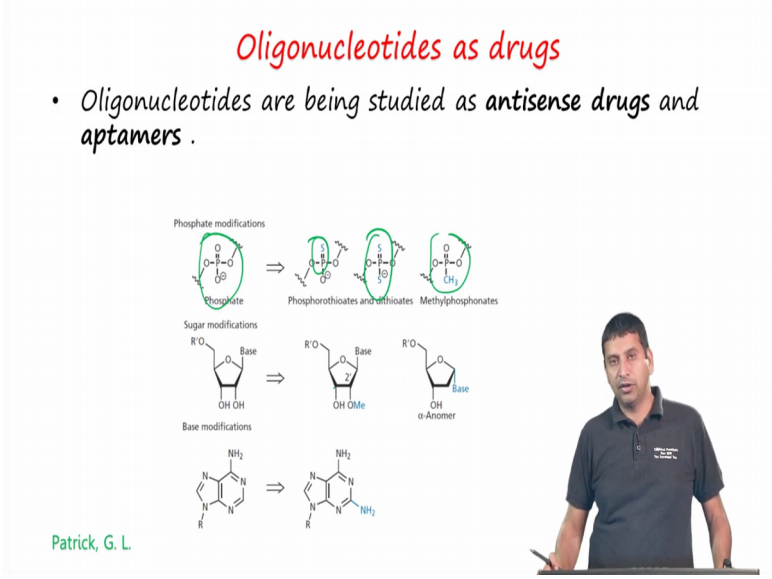

So, for example an aromatic ring can be replaced by a pyridine ring. So, here it is important that we know that this whatever change we are making does not involve major binding sites, okay. So, for example here the binding interactions with the target and the ring should remain exposed to the surrounding water medium and the Peptidomimetics is bound. Otherwise what will have to happen is that the binding site, a binding group will have to be desolvated before it goes and binds to the binding site. That is the ligand will have to be desolvated before it goes and binds to the target.

Which results in energy penalty, okay. And this would of course lead to a decreased binding affinity and loss of activity. Another potential problem with peptide lead compound is that there are invariably quite flexible molecules with a large number of freely rotatable bonds. So, a lot of studies have suggested that such flexibility is not very useful for making the molecule orally bioavailable. So, certain reunification tactics which we have already looked at previously may will be beneficial.

The next major naturally occurring molecules are nucleotides. So, oligonucleotides are being studied as antisense drugs and aptamers. So here is the normal structure of a nucleotide. So here the phosphate for example can be modified to a thiophosphate or Base thiophosphate or methyl phosphate for example. And this sort of prevents the or improve the stability and also in certain cases it can alter the binding.

Okay, you can also have the sugar is being modified and of course the basis can also be modified. So all these 3 possibilities are in play, okay. And we have already discussed

previously the roles of oligonucleotides as drugs and so we will not be spending much time here.