

hello everyone ah i welcome you all in ah bio ah molecules lecture series today we are going to discuss ah lecture eight before going to the main course of uh todays lecture i will ah give a recap to the last lecture in the last lecture we talked about ah the amino acids and there we discussed about the you know different type of amino acids based on their ah structures ah where we divided it in you know amino acids with the aliphatic side chain amino acid with the you know ah acidic side chain amino acids with the you know amides have acetic side chain that amino acids with the basic side chain that amino acids with the you know heteroaromatic cores all these you know ah amino acids with the aerial ah side chain and amino acids with the you know ah thial as a software containing side chain all these you know ah you know classification we discussed in detail ah and then we talked about ah are you know acidic and basic properties you know amino acids acidic and basic property ah and where we learned ah that ah generally amino acid has you know ah tendency to be in its you know ionic structure ah ionic form and that is you know ah in totality it is basically neutral but you know ah in the scaffold ah amine tries to be in the ammonium form whereas the carboxylic group tries to remain in the carboxylate form and ah the ph at which the population of these molecule remains you know in the ionic form ah is known as the isoelectric point so ah there we ah discussed about the you know um how ah isoelectric point ah is important and we have also ah talked about the ah how to calculate the isoelectric point that you know um you know if you know the p k of ah you know particular functional ah group in the amino acid then we can easily evaluate the isolate point which is basically if you know the two p k ah i mean the there are only two functional group in mainly ah one is the carboxylic acid and other is the ah amine ah at the alpha position so if you know the p k of these two functional group ah by adding and then dividing by two one can get the ah isoelectric point at which it tries to remain in the ionic form where amine remains in the ammonium form and carboxylic acid remains in the carboxylate form basically what happens that you know carboxylic acid proton gets transferred on the amines ah nitrogen and forms ammonium ah we also discussed about ah how to ah determine the isoelectric point ah of the amino acid with the different substituent substituents means you know if it has ah ah

proton receiving substituents like you know um heteroaromatic cores ah ij  
substituent are  
you know some you know hetero atoms are there in that case how we can evaluate  
they are also you  
know basically if it is a it has like a unusual side chain then in case of  
base we consider that  
you know ah basic group as well as the amine ah i mean p k we add and then  
divide it by two  
whereas if it is like you know acidic amino acid then you know carboxylic acid  
p k and the acids  
another substituent acids p k and and divided by two its a very simple way to  
determine we also  
discussed about protein structure in the protein structure we learned that you  
know there are four  
types of structures and in the primary structure ah protein of the protein  
basically we discuss  
about the sequence of amino acid ah in the chain and ah you know the location  
of all the disulfide  
ah bridges whereas in the secondary structure ah where regular conformations  
assumed by the segment  
ah of the proteins backbone when it falls and it comes in a repetitive manner  
in the secondary  
structure basically the secondary structures are regular conformations assumed  
by the segment of  
ah you know ah proteins backbone when it folds so ah that that determined the  
second is a tertiary  
structure you know is the three dimensional structure of the entire ah protein  
whereas in the  
quaternary structure if the protein has more than one polypeptide chain then  
what is the what way  
the individual polypeptide chains are arranged in the protein if it has more  
than one product that  
that determines the quaternary structure ah  
so ah i will start from the you know determining  
the primary structure of polypeptide chain and ah how how to determine the  
primary  
structure of a polypeptide or protein  
so how to determine the primary structure of a polypeptide or a protein  
so one way to determine the structure of a  
polypeptide that you know we can break the disulfide bridges ah in the  
polypeptide  
so that you know it can attain a basically a linear structure so  
the important i mean transformation for ah determining the primary structure  
of  
polypeptide is breaking the disulfide bridges breaking the disulfide bridges  
how to do this how one can attain the you know breaking of disulfide bridges  
its  
very simple the one way to do is reduce the disulfide bridges reduce the  
disulfide bridges this is in order to obtain a single  
perpetration reduce the disulfide bridges in order to obtain a single  
polypeptide polypeptide chain  
so how one can attain this transformation  
ah for that we will use reducing agent ah two mercapto ethanol which will ah  
you know

break the disulphide bridge by the reaction with the you know reducing is a two mercapto ethanol  
so here we are going to use a reducing agent that will basically oxidize the disulfide functional group  
so we will use we will we will achieve this transformation by applying a reducing agent agent two mercaptoethanol two two mercaptoethanol  
so let me write here  
the molecule with the disulfide bridge this is the representative you know structure of a polypeptide with the disulphide bridge this is the disulfide bridge and we will react it with two mercaptoethanol two mercaptoethanol  
so after reaction it will get converted to the corresponding sulphide will indicate terms with thial it will get ah solid sulphide will get reduced to you know ah thial  
so and tumor capture ethanol will get converted to you know sulfide now the possibility that you know if these thials remain as it is you know as we know that they have tendency to again get you know ah oxidized and get converted to disulphide  
so it will be further reacted with the iodoacid  
so protein thiol group will be treated with the iodoacetic acid  
so that it can convert it gets converted to to the corresponding two molecules of  
so now after the reaction with after the reaction with iodostic acid this is hydroastic acid this thial group you know prevents from the again disulfide bridges ah as a result of oxidation now again it can go for oxidation and form the you know disulfide but once you treat this protein thial group with the iodo acetic acid iodo stick acid sulphur ah basically oxidations gets ah you know stopped and it gets converted to the corresponding derivative  
so now it is not available for again going back to the and that way you can ah get the you know polypeptide chain without the disulfide bridges now ah  
so the this can also indicate that you know ah how many disulphide bridges are there by the number of this ah you know i mean ah substituent one can determine now lets talk about the determining the number and kinds of amino acid determining the number and kinds of kinds of amino acids to determine the number and kinds of amino acids number and kinds of amino acids in the polypeptide chain in polypeptide chain it was dissolved in very concentrated hydrochloric acid it was dissolved in six molar its very strong hydrochloric acid and heated heated at hundred degree centigrade for 24 hours basically we are here hydrolyzing all the amide ah linkages  
all the amide you know functional group to convert it into the particular you know amino acid  
the constituent amino acids which after the condensation they form the you know peptide chain  
so by treating it with a very uh strong acid that is a six molar ah

hydrochloric acid and refluxing  
it at hundred degree centigrade ah i mean hitting it at hundred degree  
centigrade not refluxing you  
know ah i will say here ah to be more appropriate ah for the 24 hour all the  
pep basically amide  
linkages gets hydrolyzed and it forms the carboxylic acid and the amine  
so in totality  
i will write here if you treat polypeptide polypeptide the six molar scl at  
degree  
centigrade 24 hour it generates amino acids the constituent amino acids the  
mixture of amino acid is passed  
through a amino acid analyzer now the mixture of this mixture of amino acid is  
passed through an amino acid analyzer amino acid analyzer to identify the amino  
acid to identify the amino acid ok now  
so in the primary structure  
as i mentioned that ah we need to know that number and ah type of amino acids  
as well as the  
ah disulphide bridges in the peptide chain these are the requirement for  
primary structure which we  
are evaluating ah by these two ah transformation first one we did that you  
know by treating the  
polypeptide chain ah with the thio merceptol ah to ah you know reduce the  
disulfide bridge into  
the corresponding thial and then ah um this ah thial since has ah you know  
kind of property  
to go again ah you know ah basically ah oxidized to the disulfide  
so again it is get  
reacted with the ioistic acid ah to ah to form the corresponding you know um  
ah stick acid analogue  
so that it cannot re ah you know oxidized to sulfur disulfide ah ah and  
so that way we we we we  
can ah easily identify in the polypeptide chain by this transformation how  
many you know disulphide  
diseases are there another thing that you know how many amino acids are there  
that to ah and what  
kind of amino acids are there that can be easily ah learned by ah treating it  
with the six molar  
hcl ah and heat it ah at 100 degree centigrade for 24 hour ah one can ah get  
the ah all the  
amino acid in the mixture and which can be easily identified through the mix  
amino acid ah  
you know analyzer that what are the amino acids are there in that particular  
polypeptide chain now  
i will talk about the you know secondary structure secondary structure  
so secondary  
structure describes the describes the repetitive conformations repetitive  
conformations assumed by segment of assumed by segments of the backbone chain of  
the backbone chain of a polypeptide protein in other words secondary structure  
describe  
the segment of the backbone fold secondary structure this crives  
how segments of the backbone how segment segments of the backbone fold segments  
of backbone fold three factors determine the  
secondary structure of a segment of protein three factors basically three  
factors determine the

secondary structure of a segment determine the secondary structure of a segment of protein segment of a protein first one the regional planarity the regional planarity linear planarity about each peptide bond about each peptide bond the original planarity about each peptide bond why is it

so as a result as a result of the partial double bond character of the amide bond double bond character of the amide bond of the amide bond which limits basically which limits the possible conformations of the peptide chain limits the possible conformations possible conformations of the peptide chain

so what are the three factors for the secondary structure that determine the you know structure of a segment of a protein first one is the regional planarity about the each peptide bond you know how ah the every peptide bond is having you know planarity and that occurs because of the double bond characteristic of the amide ah you know functional group

so that basically restricts the you know conformational possibility of the peptide chain

so that is one factor second is minimizing energy by maximizing the number of hydrogen bond minimizing energy by maximizing the number of hydrogen bond by maximizing the number of hydrogen bond hydrogen bonds between peptide groups that is hydrogen bonds between the carbonyl oxygen hydrogen bond between carbonyl oxygen oxygen of one amino acid of one amino acid and the

amide hydrogen of another hydrogen of another

so how does it form let me through the structure i will represent here

so as i mentioned carbonyl of one here i am putting the carbonyl group and amide of other

so this is one strand and the other strand is

so this is the as i mentioned you know ah carbonyl of one you know amide chain with the amide of the other you know amid hydrogen of the other these forms the hydrogen bond ok

so hydrogen form hydrogen bonding between peptide groups peptide groups hydrogen

bonding between peptide groups ok now the ah third thing is the need of adequate separation the need for adequate separation between the neighboring r group adequate separation between neighboring r groups neighboring r groups to avoid steric strain steric strain and repulsion of like charges of like charges

so the need for adequate separation between neighboring r groups to avoid steric strain and repulsion of like charges

so again i will like to repeat the secondary structure secondary structure describes the repetitive conformation assumed by segment of the backbone in other words that secondary structure describe how segments of the backbone fold segments of the

backbone fold and to learn about it you know we need to know the three factors you know ah which

determine the you know structure of a segment of a protein what are the three

factors first one is the regional planarity about each peptide bond and that how does it happen because of the you know double bond characteristic double bond characteristic of the amide bond which limits the possible conformation of the peptide gene because you know if the double bond characteristic comes in because of the amide group it does not allow that flexibility which can occur in its absence so the conformational flexibility is lost in the peptide chain because of the double bond characteristic second is minimizing energy by maximizing the number of hydrogen bonds as we know that this is the nature law that every you know entity in this nature tries to remain in the least energy state and ah to achieve that you know all kind of physical processes happens and here basically the because of the hydrogen bonding you know its ah because of the hydrogen bonding its minimizes its energy you know ah and that can easily occur in the peptide chain ah particularly in between the you know carbonyl group of i mean oxygen of the carbonyl group and the ah amidic nh you know ah with the ah you know ah hydrogen bonding can takes place so ah carbonyl oxygen of one ah you know ah i mean m i d group and the nh of the another mid group can engage in the hydrogen bond ah that is very clear from this you know schematic presentation how how this carbonyl oxygen and the other ah amid nh are engaged in the hydrogen bonding also the need for adequate separation between neighboring r groups to about steric strain so that you know it cannot you know have a steric so ah the folding takes place in a way ah so that the all the you know neighboring ah r groups ah remain far apart to have the minimal ah energy state ah and to about the sterics so these are the you know ah to add the steric repulsion basically so ah ah also ah if charges are there like you know as we know that you know um amine can be in the ammonium form or the side chain can be in the ionized state so there also they need to remain far apart so that they cannot have you know ah repulsion between ah each other ah if the like charges are there then they can have like repulsion so ah this this is the the third ah you know um ah factor ah now ah based on that i will like to ah talk about the you know ah you know different type of secondary structure

ah first one is alpha helix alpha helix alpha helix one type of secondary structure is

an alpha helix this is one and in alpha helix in alpha helix the backbone of the polypeptide the backbone of the polypeptide coils around the long axis of the protein molecule coils around the long axis of the protein axis of the protein molecule coils around the long axis of the protein molecule

so in alpha helix the backbone of the polypeptide coils around a long axis of the protein molecule ah how does it happen to represent it let me draw the structure

so this is the polypeptide chain  
so this is the coiling and  
since it is doubly extended  
so basically the polypeptide chain is coil around this axis the long axis of the protein molecule now because the amino acid have the l configuration because the amino acid have the l control the alpha alex is right handed amino acid have the al configuration the alpha alex is a right handed helix alpha helix is a right handed helix right handed helix that is it rotates in a clockwise direction that is it rotates in a clockwise direction clockwise direction as it spirals down

so this is the clockwise  
so basically its coming  
so this is the clockwise as it you can see  
you can see here like as it spirals down eastern of the helix contain 3.6 amino acid this eastern each turn of the helix contains 3.6 amino acid contains 3.6 amino acids and the repeat distance of the helix is five point four angstrom you can see here the repeat distance of the helix is five point four angstrom five point four angstrom and three point eastern of the helix content three point six amino acid exactly and basically it has the hydrogen bond as i told that you know in the secondary structure basically four amino acid away this hydrogen bonding take is taking place four amino acids away four amino acid away and the repeat distance of the helix so each turn of the helix contains 3.6 amino acids and the repeat distance of the helix and the repeat distance and the repeat distance of the helix repeat distance of the helix is 5.4 angstrom this is important information basically now let us talk about

so this is one alpha helix is the one ah secondary structure and the second second one is beta pleated seed beta pleated seed the second type of secondary structure is beta pleated sheet how does it look like in the bleed operator sheet i will make the pictorial ah representation through which you will understand so in the beta pleated sheet two seats are anti parallel

so let me draw first anti parallel as i told you  
so that is why i am you know making this opposite orientation of these two seeds and basically these are the two seeds just i am

with the solid lines i am representing  
that the two sheets are there which are folded in anti parallel to each other  
so this  
is a anti parallel beta pleated sheet and then i will draw here the other  
so other secondary structure the  
polypeptide chains are parallel parallel  
so this is the parallel beta pleated sheet  
so parallel beta pleated sheet here the hydrogen bonding in a beta pleated sheet  
in a beta pleated sheet occurs between neighboring peptide chains  
these are the two peptide strands  
so basically this is the you know in between the hydrogen bonding is taking  
place similarly this is  
the anti parallel and in the case of parallel also i can represent here  
so the hydrogen bonding in a beta pleated  
sheet occurs between neighboring peptide chain between neighboring peptide chains  
between neighboring peptide chains a beta pleated sheet is almost fully extended  
a beta pleated sheet is almost fully extended and the average two amino acid  
repeat distance is seven angstrom average two amino acid repeat  
distance is submitting a strand the part of the backbone is structure of a  
protein  
background that exist in a beta pleated sheet the part of the backbone structure  
backbone is structure of a protein background that exist in a beta pleated sheet is  
see it is indicated by indicated by a flat arrow pointing  
so this is i am talking about the flat arrow  
flat arrow pointing in the end to c direction flat arrow pointing in the end to  
c direction again i will repeat here in the beta pleated sheet the hydrogen  
bonding in a beta pleated sheet  
between the neighboring peptide chains occurs that i have shown here in these  
two cases you know in one case it is anti parallel  
another case the you know parallel  
so a beta pleated sheet is almost fully extended the  
average two amino acid repeat distance is seven angstrom and the part of  
the backbone structure  
of a protein background that exist in a beta pleated sheet is indicated by a flat  
arrow that that is  
the flat arrow pointing in the end to c direction for example silk contains a  
large  
proportion of relatively small amino acids silk contains a large proportion of  
relatively small amino acid relatively small amino acid and therefore has large  
segments of beta pleated sheet therefore has large segments of beta pleated  
sheet of beta pleated sheet the number of side by side is strained in a beta  
pleated sheet ranges from two  
to fifteen in a globular protein side by side strain i am particularly this  
talking about this side by side strain the number of side by side  
strains in a beta pleated sheet in a beta pleated sheet ranges from two to fifteen  
in a globular protein globular protein the average strain in a beta pleated  
sheet section contains six amino acid average is strained in a beta pleated c  
sheet section contains six amino acid  
so these are the another wool and fibrous portion of  
the muscle have secondary structure fibrous protein of muscle wool and fibrous  
protein of muscle have secondary structure that are  
almost all alpha helix alpha helix beta pleated sheets are ordered  
in the silk and spider web are occurred in silk and spider web we are seeing  
spider web in our houses also and

you know spider wave ah and it cannot be staged cannot be stretched  
so i will stop here reelector  
and i will again continue with the you know um structure of protein ah  
particularly the secondary  
structure in the next class thank you very much you

Prutor@IITK