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Subject :	ELISA
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What we should remember about ELISA :

-it's an immune assay which involves antigen-antibody reactions .

-enzyme linked : the secondary antibody is attached to an enzyme which has the capacity to convert the colorless or even the colored material into a new color provided if the antigen (protein) is there .

-pregnancy test is an ELISA method .

Protein sequencing

What do we mean by protein sequencing?

-is the process of knowing the order of amino acids in a protein or a peptide.

So what the benefit of knowing these sequences?

-identify the protein .

-knowing the structure then the function which helps to design drugs to affect the process where this protein is involved ." you don't know the structure , you can't do anything! "

How we can know these sequences?

-by their gene : by knowing the codon (every three nucleotides)which represents an amino acid (we can know the gene sequence from the DNA or mRna).

-you have to return the protein to its primary structure (**denaturation**), after we have an aliphatic structure we can use a tag(**PITC:phenylisothiocyanate**) which attach to the N-terminus (the free amino group which is the only one we have), then we use an

Exopeptidases (an enzyme that have a capacity of breaking down only one amino acid at a time from the N-terminal), finally you achieve one amino acid at a time , then you break the second amino acid .. third and so on . this procedure called **Edman Degradation** .

(by hydrolysis you can know the constituents of this protein but not the sequences.)

This procedure is very lengthy , costly and it is not efficient for peptides with more than 50 amino acids . So if we have a protein with very long sequence what should we do ??

Simply we can cleave this protein into smaller peptides then applying edman degradation to these peptides . we can cleave the protein by two methods :

1-using enzymes :

a) **Endopeptidases** : enzymes that cleave at specific sites <u>within</u> the primary sequence of proteins. These enzymes differ in their specificity (not all of them cleave the same peptide bond).

Examples : Trypsin and pepsin (these are a natural enzymes that we can find in our digestive tract to digest the proteins).

Ex : Trypsin : cleaves polypeptide chains on the <u>carboxyl side</u> of arginine and lysine (positively charged amino acids) .

Q- if you have a chain of amino acids and you apply an enzyme cleavage then get an amino acid pieces ,

-if you use trypsin and get two pieces , what this means? This means that this chain has at least one Arg or one lys . If you get three pieces? Means there is two Arg or two lys or arg and lys.

b) **Exopeptidases** : enzymes that cleave amino acids starting at the end of the peptide, they are two types :

***aminopeptidases** that cleave at the N-terminal.

***carboxypeptidases** that cleave at the C-terminal.

2- chemical digestion :using chemical reagent that cleaves peptide bonds by recognition of specific amino acid residues , the most commonly used is **cyanogen bromide (CNBr),** it cleaves at the C-terminal side of methionine residues.

-a protein that has 10 Met residues will usually yield 11 peptides on cleavage with CNBr.

After cleavage of protein by chemical digestion or enzymes we continue sequencing by Edman degradation .

How we can determine the structure of the protein ?

1-crystallography

Used to determine the 3D structure of a protein. Mechanism : After making sure that you have a very pure sample which contains only one protein (this achieved by electrophoresis, if it gives us only one band then we can say that the sample is pure) then having a well with covering plastic slide and put up a drop of the protein ,after slow evaporation of water and adding some materials you will get a solid structure (knowing the mechanism not specifically required). Finally you have the protein with crystal like structure then exposed it to X-rays which scattered by the electrons of the molecule(every protein reflects the X-ray different from other proteins because it contains

different molecules), then computerized to do calculations and detect the amino acids then the 3D structure.

The advantages : very clear technique to visualize proteins but it measures the structure in a solid state (crystals) while the protein is behaving usually in solutions ,so proteins might behave differently in solutions than crystals , accordingly functional wise has many Criticisms (structural wise , it is the best way to visualize proteins) .

2- Nuclear magnetic resonance (NMR) spectroscopy

It can measure the structure of a protein in a solution by magnetic resonance technique (an electrical field affecting structures causing the electrons inside to spin clockwise or counter clockwise), because nuclei differ in different atoms they respond differently to the electric field by this we can determine these atoms then you can know the amino acids .it's hard to visualize the protein and it's not really as accurate as crystallography .

Enzymes

To define enzymes we have to mention that :

1-they are proteins which works as catalyst(catalyst means speeding up chemical reactions).

2-not being consumed (specifically they are changing during the reaction , and at the end of the reaction they return to their first state).

3-they are specific to their binding material which is called substrate.

4- they are usually used in small amounts because they reused.

Tow properties of a protein characterize its interaction with ligands :

-Affinity : the strength of binding between a protein and other molecules (or how much the attraction between the substrate and the active site).

-specificity : the ability of a protein to bind one molecule in preference to other molecules. (general speaking that all enzymes are specific but that does not mean that the enzyme bind to only one molecule it's may bind to more than one molecule , so enzymes vary in their specificity)

Example : Hydrogen peroxide H2O2 is a toxic material but it's produced inside our bodies which get rid of it by an enzyme which called catalase (has a five coordinated heme) , this enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen. This reaction can occur by itself but need lot of time, so the aim of using enzymes is to speed up the reaction resulting in preventing accumulation of this toxic material inside our bodies . (H2O2 produced in high amounts).

Enzymes accelerate reactions in range of 10^6 to 10^14 up to 10^20 , e.g. catalase can convert a hundred million H2O2 molecules into water and oxygen in one second (it is a very efficient enzyme ,why? Because H2O2 is a toxic material)

*The enzymes that deal with toxic materials are designed to be very processive and very efficient to prevent accumulation of this material within the body.

*Enzymes differ from chemical catalysts (enzymes are much faster).

Why do chemical reactions occur ?

Because all materials tend to be stable , (general speaking by nature almost they are stable because we can deal with it , but they are looking to achieve higher stability).