Enzymology L1

The first question, what’s the enzyme, and what are the characteristics of the protein molecules that are called enzymes

Enzymes are organic catalysts that function to accelerate the reaction

Reactions can occur without enzymes but they’ll take a very long time

In addition to the organic catalysts there’s what’s called the inorganic catalysts, eg: metal ions.

Example: in the hydrogenation reaction that converts oils into fats, a metal is used to accelerate it. ^\_^

Enzymes are never consumed nor undergo permanent alteration as a sequence of the reaction they catalyze. When an enzyme molecule catalyzes a reaction it’s not consumed and it leaves the reaction to catalyze another reaction as long as the life span of the enzyme is not over.

But what’s the life span of the enzyme?

Each protein molecule –including enzymes- have what’s called a life span

Usually, the proteins that perform a dynamic function (globular proteins) have a short life span which is about few seconds

Let’s suppose that an enzyme has a life span of 155 seconds, it will start catalyzing a specific reaction and won’t be consumed and won’t undergo permanent alteration until the 155 seconds are over. ^\_^

So, each enzyme leaves the reaction after converting the substrate into product to catalyze another reaction and giving another product molecule and then again leaves the reaction without being consumed or altered to catalyse another reaction and so on until its life span is over

In other words enzymes are not a part of the reaction (it’s not a substrate and it functions as a catalyst)

All enzymes are protein in nature except for one type that’s called the ribozyme

Ribozyme: short segment of RNA molecule –about 90 to 300 nucleotides- that behaves as an enzyme and is responsible for the catalytic processing of the RNA molecules

تفسير للكلام:

The nucleic acids are 2 types (DNA and RNA):

There are 2 types of DNA

The 1st is the nuclear DNA which is found in the nucleus and it’s made out of 3.5x109 nucleotides

The 2nd is the mitochondrial DNA which is found in the mitochondrion and is made out of 1.6x104 nucleotides

On the other hand there are lots of types of RNa

-mRNA(messenger RNA)

-rRNA(ribosomal RNA)

-tRNA(transfer RNA)

-siRNA(small interfering RNA)

-hnRNA(heterogeneous nuclear RNA)

-microRNA

-ribozyme ^\_^

When any type of RNA is made it’s immature (non-functioning) and in order for the RNA to function it must be converted from the immature form to the mature functioning form

The enzyme that’s responsible for this conversion is the ribozyme

This sentence is related to what’s called the turnover umber

Turnover number: it’s the number of substrate molecules that can be converted by one molecule of an enzyme into a product molecule in the unit of time (1 second)

In other words, if an enzyme molecule is put in the reaction, how many substrate molecules it will convert into product in 1 second.

Generally, the enzymes have a high turnover number which is about 106 to 1012

On the other hand the inorganic catalysts (metal ions) have a small turn over number which is about 103

Note: highly efficient catalyst has a high turnover number. So, enzymes are highly efficient.

Enzymes are selective catalysts –this is the most important characteristic of enzymes- and it means that each enzyme acts only on one substrate

With too little exceptions that some enzymes can act on two substrates

Enzymes are thermoliable, it’s because they are proteins

The structure of proteins:

1-primary structure: it’s simply the amino acids bound together by peptide bonds in a linear form

2-secondary structure: it’s the alpha helices and beta sheets and loops that that are formed because of the hydrogen bond interactions between the back bone of the amino acids

3-tertiary structure: it’s the complete, final and functioning 3D structure of the protein and it’s formed due to the electrostatic interactions(ionic bonds), disulfide bonds and hydrophobic interactions

4-quaternary structure: it’s the same as tertiary but the structure is called quaternary when the protein is made out of more than one polypeptide chain that are not bound together by covalent bonds

The peptide bond is the strongest bond in the protein structure and the disulfide bond is the second

The term thermoliable means that in high temperatures the protein will be denanturated (loses its 3D structure):

The denaturation is the breakdown of the quaternary, tertiary, and secondary structure back to the primary structure (the enzyme loses its 3D structure and becomes linear) and this leads to the enzyme losing its function

Enzymes are site specific: (compartmentalization of reaction)

Reactions that take place in the cytosol are different from the reactions that occur in the mitochondria are different from the reaction that take place in the nucleus,

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The enzymes that are present in the cell membrane are not the same as the enzymes that are presents inside the mitochondria and so on

Nomenclature of enzymes:

1-addition of the suffix “ase” to the substrate that the enzyme functions on.

Examples:

The enzyme uricase acts on uric acid

The enzyme urease acts on urea

But this name doesn’t give information about the type of reaction that’s being accelerated by the enzyme (oxidation, reduction…etc)

So, the name of the enzyme should be given to according to the reaction it accelerates.

Examples:

Oxidase: oxidation

Reductase: reduction

Methylase: methylation

Phorphorylase: phosphorylation

But this name doesn’t give information about the substrate that’s being acted upon

So, the name of the enzyme should include both the name of the substrate that will be acted upon by the enzyme and the type of reaction that’s being catalyzed by the enzyme

Example:

Lactate dehydrogenase works and the substrate lactic acid and accelerate the dehydrogenation reaction of it (oxidation) ^\_^

Please note that some enzymes still have their old traditional names, such as: catalase, pepsin, chymotrypsin, trypsin and amylase. And all of these enzymes are digestive enzyme (hydrolases) –بنشرحه لقدام-

Classification of the enzymes:

The (IUBMB) has put a system that classifies the enzymes into 6 classes and each enzyme in these classes is given a code number that’s made out of 4 digits

This code number is called Commission Numerical Code (CNC)

The first digit is the class digit and it is given according to the major class of the enzyme (one of the 6 major classes)

Oxidoreductase = 1

Transferase = 2

Hydroxylase = 3

Lyase = 4

Isomerase = 5

Ligase = 6

The second digit is the subclass digit and it is given according to the type of the sbustrare acted upon or the bond to be cleaved

The third digit is the sub-sub class digit and it’s given according to the functional group that’s acted upon by the enzyme and the cofactor that will function with the enzyme ^\_^

The fourth digit is the number of the order in which this enzyme is added to the list

The first enzyme discovered is given number 1 and the second is given number 2 and so on, and all of them belong to the same class

Classes of the enzymes:

Oxidoreductase:

In human body, the oxidation reaction never takes place without being combined with a reduction reaction; because oxidation is the removal of hydrogen from the substrate, which will increase [H+] in the media and converts it into and acidic media which will stop the activity of the enzyme ^\_^

So, it must be given to a “carrier”

Example: the lactate dehydrogenase enzyme activates the oxidation reaction of lactic acid and removes 2 hydrogen atoms from it

In this reaction, the carrier that accepts the hydrogen atoms is NAD+ (Nicotinamide adenosine dinucleotide)

So, when lactic acid is oxidized, NAD+ is reduced to NADH + H+

So, the oxidation of a substrate is always coupled with the reduction of another substrate

Quick note:

The enzyme that carries the number 1.1.1.1 is called alcohol dehydrogenase and its number is explained as the following

First digit indicates that the enzyme is from the oxidoreductase class

Second digit indicates that the substrate the enzyme acts upon is alcohol

Third digit indicates that the cofactor of the enzyme is NAD+

The fourth digit indicates that this enzyme is the first enzyme to be added to this category

2-transferases:

They transfer a functional group from a donor to an acceptor

Example:

The enzyme Phenylethanolamine N-Methyl Transferase (PNMT) converts norepinephrine into epinephrine by adding a methyl group to it

It’s called methyl transferase because it transfers the methyl group from the donor to the substrate

The methyl group donor of the most methylation reaction in the body is S-Adenosyl Methionine (SAM)

When the methyl group is taken from SAM it’s given to norepinephrine, which converts it into epinephrine.

The removal of the methyl group from SAM converts it into S-Adenosyl Homocysteien (SAH)

3-Hydrolases:

They cleave the bond by adding a water molecule to it, eg: the digestive enzymes

Starting from amylase in the mouth that breaks the glycosidic bonds (a-1:4) and (a – 1:6)

All the digestive enzymes are hydrolysaes

Eg: the enzyme lipase that’s secreted from the pancreas breaks down TAG by the addition of 3 molecules of water to breakdown the 3 ester bonds in TAG to produce 3 F.A. + glycerol

4- lyases:

Adding a group to breakdown a double bond or removing a group to create a double bond

Eg: fumaric acid contains a double bond, by the addition of a water molecule it will be saturated

Both hydrolase and lyase use water molecule in this case

The hydrolase adds a water molecule and cleaves the substrate

But the lyase adds the water molecule without cleaving the substrate

5-isomerase:

It converts one molecule to another through the intramolecular rearrangementsn

Example is the conversion of 3-phosphoglycerate to 2-phosphoglycerate, the phosphate group is moved from the carbon atom number 3 to the carbon atom number 2

6-ligase: (C-C, C-S, C-O, C-N)

A bond is made or broken

The most important is T4 DNA ligase –which is the only enzyme that consumes energy while functioning-

Active site: it’s the site in which the substrate binds

In order for the reaction to occur, the substrate must bind to the enzyme in the active site

Sometimes the active site is called the catalytic site

It is made out of a small segment of the protein(enzyme) structure and it’s the pocket/cleft that the substrate binds to

Characteristic of the active site:

1-the active site should have a specific 3D configuration

2-it should be complementary in shape to the substrate

3-it should contain some specific groups (highly reactive amino acids)

The highly reactive amino acids contain a hydroxyl group, such as: serine, threonine, tyrosine and hydroxyproline

The amino acid that contributes more than the other amino acids in the formation of the active site is serine

Because tyrosine is used in the production of catecholamines that are secreted from the adrenal medulla and in the formation of the hormones that are secreted from the thyroid gland (T3+T4) and it participate in the production of melanin so it won’t be available to be used in the active site formation

Threonine is also not available because it’s an essential amino acid

Hydroxyproline is not available because it’s a modified amino acid

Serine is a non-essential amino acid, so it’s synthesized in body and it’s available

Also, the sulfur containing amino acids are highly reactive such as cysteine, to form a disulfide bridge with the substrate

Also, the acidic amino acid (aspartate and glutamate) are highly reactive

Also, the imidazole ring of histidine is highly reactive

The binding of the substrate in the active site should be stabilized in order for the enzyme to do its function

This stabilization needs the presence of functional groups in the binding site of the substrate and reactive sites groups in the active site of the enzyme to bind the substrate to the active site via the weak interactions (hydrophobic bond, hydrogen bond, disulfide bond, ionic bond) which catalyzes the reaction

The arrangement of the functional groups in the active site is responsible for the specifity of the enzyme to specific substrate (if we take a 3D picture to the active site of the enzyme lactate dehydrogenase we will find that’s its different from the active site of the enzyme pyruvate dehydrogenase)

If the enzyme consists of two polypeptide chains (made from more than one monomer), then the active site will be in the interface between the two monomer recruiting the presence of the functional groups from each monomer which leads to the substrate being more stabilized because the binding will be formed between the functional groups on the first and second polypeptide chains,

Now, how the substrate is going to bind to the active site of the enzyme?

This question is answered via two models:

1-lock and key model:

The 3D structure of the active site is complementary to the configuration of the substrate and the binding site of the substrate

The model is rigid and unable to be changed and won’t undergo any dynamic changes (if the active site of the enzyme is not complementary to the binding site of the substrate, no bond formation will happen and no reaction will occur)

If there’s alteration in the active site or in the binding site there will be no reaction

2-induced fit model:

The active site of the enzyme and the binding site of the substrate are not complementary to each other and once the substrate gets closer to the active site of the enzyme, the active site will undergo conformational changes to be suitable to fit to the binding site of the substrate.

Although there is no complementarity between the binding site of the substrate and the active site of the enzyme, when the substrate comes close to the active site the modification happens and the active site becomes fit to the binding site

Once the reaction is over and the substrate is converted into product the active site returns back to its original form

This doesn’t take place in the case of enzymes alone, there’s also factors like hormones and proteins which can induce these conformational changes. Also this model is not rigid (it’s flexible) and it can undergo some dynamic changes

Mechanism of action of enzymes:

firstly the Substrate binds to the active site and forms enzyme-substrate complex and this reaction is reversible reaction, so it has two constants:

K1 is the constant for the forward reaction which allows the binding of the substrate to the active site to form the enzmye(association constant)

K-1 is the constant that allows the substrate to be dissociated from the active site of the enzyme (dissociation constant)

supposing that the enzyme-substrate complex is formed, the complex will allow the conversion of substrate into product, so the final products of the reaction are the product molecule and the enzyme molecule which goes to catalyse another reaction.

this reaction also is reversible and has two constants:

K2 is the constant for the forward reaction (formation of the product)

K-2 is the constant for the breakdown for the ability of the enzyme to convert the substrate into product

so, the reaction catalysed bt an enzyme has four constants:

K1 for the the association between substrate and active site of the enzyme

K-1 for the dissociation of substrate from the acitve site of the enzyme

K2 for the conversion of subustrate into product

K-2 for the breakdown of the ability of the enzyme to convert substrate into product

the least bonstant is the K2 because the enzyme has high tencency for completing the reaction and forming a product and too little enzyme-substrate complex will not be able to be converted into enzyme and product and thus the least constant is the K-2

the mechanism of enzyme activity can be explained by two prescriptions:

1-thermodynamic changes:

compairing the catalysed reaction to the uncatalysed reaction, we would find that in both reactions, the substrate and the product energy are the same

the difference is in the energy of the transitional state and it has a higher energy in uncatalysed reaction

ΔG (activation energy) is the difference between the energy of the substrate and the energy of the transitional state and it's higher in the uncatalysed reaction

توضيح:

after the substrate binds to the enzyme, in order for it to be converted into product it must be converted into transitional state and then it's converted into product

let's imagine that when the substrate binds to the enzyme, there is a barrier and it prevents the substrate from being converted into a product

jumping over this barrier is provided by the energy of activation (ΔG) and it allows the conversion of the substrate into product

in the case of the uncatalysed reaction the ΔG is high and in the catalysed reaction the ΔG is low

the enzyme lowers the ΔG neede to convert into product so that the barrier can be crossed easily

low ΔG means more substrate molecules that have the low ΔG that is needed for the conversion

example: the lactose sugar formed from glucose and galactose bound together by glycosidic bond

if few grams of lactose were taken and put into a test tube without any enzyme added and left for 100 years (long time), the sugar will be hydrolysed into glucose and galactose

catalysed reaction by lactase allows for faster cleavage of the glycosidic bond in a shorter time because it reduces the ΔG so most of the lactose molecule will have the ΔG

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2-Processes at the active site:

one of these four processes may occur at the active site:

a-catalysis by proximity:

supposing that two substrate molecules have 1 meter of a distance between then and another two substrate molecules have 5 cm of a space between them

the reaction that's going to take place will be between the substrates that have 5 cm between them (the shortest)

because in order for the substrates to react together they must come close to what is called the "bond forming distance" in order for the bonding to occur

when a substrate binds to active site it binds due to the formation of what's called the local region of high substrate molecule

توضيح:

not only one molecule of substrate goes close to the active site of the enzmye. Around one active site, approximately around one million molecules of substrate are found, and the 1st molecule that binds to the active site will be converted into product

so, there's croudness of the substrate molecules around the active site in what we call bond forming distance so that reaction can occur

the importance of this croudness: the high number of molecules that get close to the active site of the enzyme enhances the pushing of one molecule of substrate to the active site and this process allows the orientation of the functional groups of the binding site of the substrate to be very close to the functional groups of the active site of the enzyme to allow the formation of bonds between the substrate and the enzyme and thus catalysing the reaction.

b-acid base catalysis:

depends on the presence of the ionizable functional groups in the active site (functional gruops of histidine, glutamic acid and aspartic acid) which act as acids and bases to allow the ionization of the other functional groups and allow binding between the enzyme and substrate

c-catalysis by strain:

this procsess is specialized for the lytic enzymes (enzymes that lead to the break of a bond) e.g: the digesting enzmyes that asre found in GIT

example:

the enzyme lactase enzyme makes the breakdown of the glycosidic bond in the lactose sugar.

these lytic enzymes function by binding to the substrate in a position that's unfavourable to the bond which makes its breakdown much easier

d-covalent catalysis:

formation of covalent bond between the enzyme and one or more substrate molecule allows for the reactiom to go in an alternative pathway with lower activation energy that leads to the production of the same product

Types of enzyme specificity:

1-absolute specificity: the enzyme acts only on one particular substrate

2-group specificity: the enzyme acts on a group of molecules containing a specific functional group

eg: methylase enzyme takes a methyl group from the donor and gives it to any acceptor (not necessary epinephrine)

3-linkage specificity: the enzyme acts only on a specific bond regardless of the rest of the molecule

example: the TriAcylGlycerol (TAG) is made out of 3 fatty acid molecules and 1 molecule of glycerol

the esterase enzyme only functions on the ester bond between the fatty acid and the glycerol molecule regardless of the glycerol or the type of the fatty acid

4-stereospecificity (optical specificity): the enzyme acts only on one optical isomer

-the most specific types of specificity are the 1st and the 4th types-

lastly we have to identify the dual enzymes -it's not a type of specificity-

dual enzyme: is an enzyme that can act on two different substrates catalysing the same reaction for them or acting on two different substrates catalysing two different reactions

Done by:

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