ENZYMES:

GENERAL LOOK UPON ENZYMES:

***Material and Function:**

-Enzymes help speed up chemical reactions in the human body

-They bind to molecules and alter them in specific ways

-They are essential for respiration, digesting food, muscle and nerve function, …

-They can be either RNA (ribozymes) **or** proteins

NOTE:

Enzymes are specialized proteins, but not all proteins are enzymes

-They don't get consumed during the reaction (their concentration doesn't change)

NOTE:

Any molecule in the body gets consumption which means degradation, but here we are discussing with respect to the reaction itself

-Needed in small amounts

***Types of Catalysts:**

-Catalysts can be either:

- Chemical, **i.e.** Pt (Platine), Fe (Iron), Cu (Copper)
- Biological, **i.e.** Enzymes

NOTE:

We can compare different types of catalysts by looking upon the **relative rate**; which indicates how much moles of the reactants are consumed (or how much moles of the products are produced) per second

Example:

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2\,(g)$$

Reaction Conditions	Activation Free Energy kJmol^{-1}	Activation Free Energy kcal mol^{-1}	Relative Rate
No catalyst	75.2	18.0	1
Platinum surface	48.9	11.7	2.77×10^4
Catalase	23.0	5.5	6.51×10^8

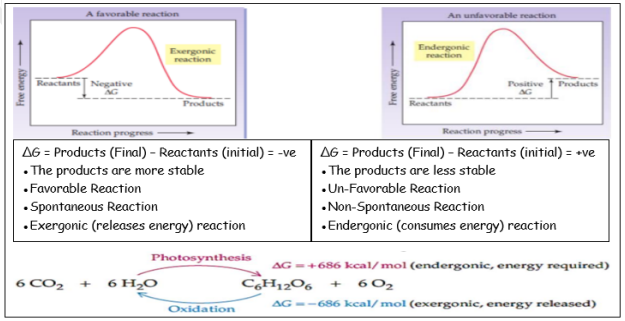
Depending on the figure, enzymes (biological catalysts) are the most efficient catalysts among all other catalysts

→ Non-enzymatic catalysts (10^2 to 10^4)-fold increase

→ Enzymatic catalysts (10^6 to 10^{14})-fold increase

i.e. catalase – 10^8 / carbonic anhydrase - 10^7

***Energy and Biochemical Reactions:**



NOTE:

-We have both reactions in our bodies

-Enzymes do not change the favorability or spontaneity of the reaction

[E + S ↔ ES ↔ EP ↔ E + P], where;

E = Enzyme / S = Substrate / P = Product

-The relationship between exergonic – exothermic or endergonic – endothermic:

All exergonic reactions are exothermic; at low temperature

All endergonic reactions are endothermic; at low temperature

At high temperature, they become the opposite, since; $\Delta G = \Delta H - T\Delta S$

-The difference between ΔG, ΔG° is that ΔG° indicates standard conditions, while ΔG doesn't

◊ Theories Concerning Reactions:

- **The kinetic theory (rate):** it understands the reaction during the pathway without looking at the starting and ending points. And this is what enzymes do. [They don't play with the favorability of the reaction]
- **The thermodynamic theory (favorability):** concerned with the energy level within the reactants and products, without giving any concern to the road (pathway) in between reactants and products; it takes only the two points in consideration.

APPLICATIONS USING ENZYMES:

***شو بفرق الشاي الأسود عن الشاي الأخضر؟**

Crushed leaves are exposed to the oxygen in air, a polyphenol oxidase (it oxidizes polyphenols) breaks up polyphenols into tannins; which are darker in color with a different taste

***كيف الأشياء الزاكية بكون إلها قوام و طعم حلو؟**

By the addition of **corn syrup**; which is produced by enzymes

***كيف بتكون الشوكولا قاسية من الخارج و طريّة من الداخل؟**

By using **sucrase**, a yeast enzyme that breaks sucrose into its monomers

***كيف يتم تصنيع الأموكسيسيلين (أكثر المضادات الحيوية شيوعًا)؟**

By using certain enzymes

***ليش لحمة المولات أطرى من لحمة الأضاحي؟**

Because of the addition of certain enzymes, **i.e. pectinases**, which breaks bigger molecules into smaller ones; thus, more soluble

***من الإنزيمات المستخدمة بعدسات العين اللاصقة و المختبرات و أوية الغسيل و غيرها؟**

Glycosidases, lipases, …

STRUCTURE OF ENZYMES:

***Active Sites:**

- A specific 3D shape, looks like canals/ clefts or crevices
- Never exists on the surface of an enzyme, they always exist inside it
- Includes a region where the biochemical reaction takes place.
- It comes from different amino acids which must not be in sequence, because they are coming from different parts of protein itself [usually forming a domain made of multiple secondary structures]

NOTE:

Enzymes structures are made up of amino acids which are linked together via peptide bonds in a linear chain

-Contains a specialized amino acid sequence that facilitates the reaction

-Within the active site are two sub-sites, the **binding site** and the **catalytic site**. If the active site was small in size, they are the same molecule, if not then they are different. For example;

if the active site is large in size, we found inside it a place for Phe - Asp - Ser

Phe: hydrophobic → responsible of binding

Asp-Ser: polar → they interact as H^+/e donor or acceptor → responsible of catalysis

-Water is usually excluded after binding unless it participates in the reaction

-The active site takes up a relatively small part of the total volume. The main function for **the rest of the enzyme** is providing support (structural role) for the active site. In addition, it provides regulation

-Extra amino acids help create the 3D active site, and in many enzymes, may create regulatory sites

-Some substances may bind at **the rest protein structure**, changing the conformation of the enzyme or the active site;

- Activator: it induces a conformational change and increases the affinity
- Activator: it induces a conformational change and decreases the affinity (causes **blockage**)

-Enzymes are very specific toward their substrates, but they have different degrees of this specificity; certain enzymes only accept one certain reactant, other enzymes can take 2 or 3 different substrates at the same time and others can take them at different times) depending on the particular chemical reaction.

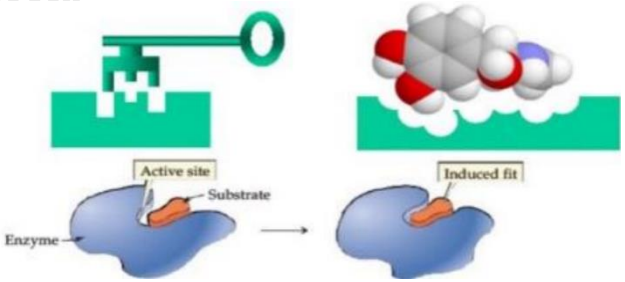
i.e. catalase only react with H_2O_2 as a reactant

glucokinase reacts with glucose and ATP at the same time, and in different sets it can react with fructose and ATP

◊ Substrate Binding at the Binding Site:
RECALL:
What is the thing that distinguishes proteins from carbohydrates and lipids?
Proteins are the only molecules which can change their shape upon binding other molecules
-Substrates are bound to enzymes by multiple weak attractions (*electrostatic, hydrogen, van der Waals, hydrophobic, & ionic*). **The initial binding** between the substrate and the active site *should not be covalent*
NOTE:
Any material binds initially the active site covalently, it will be either a toxin, poison, nerve gas or drug act as a covalent inhibitor, and it is not physiological
-Binding occurs at least at three points (chirality). Enzymes differentiate between different isomers, so if it catalyzes the reaction for D- isomer it won't catalyze the reaction for L- isomer (it won't catalyze the reaction for both enantiomers)

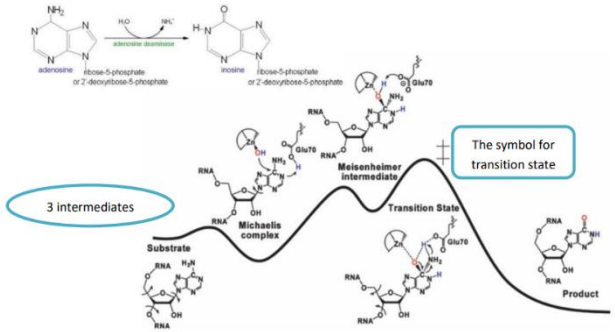
HOW DO ENZYMES WORK?
***From an Energy Point of View:**
Enzymes decreases the amount of energy needed to overcome the energy barrier (**activation energy**)

***From a Theoretical Point of View:**
◊ Lock and Key Model [The First and Not-Adopted Model -Anymore-]:
Each enzyme has a fixed shape and binds only one specific substrate at a time
Why is it Wrong?
→ Enzymes are proteins and proteins are not static in solutions
→ Enzymes can have more than one substrate, **i.e.** glucokinase
◊ The Induced Fit Model [The Second and Adopted Model Now]:
After the initial binding of the substrate with the active site, the active site changes its shape and the substrate orients itself within the active site to the best fit



NOTE:
-Enzymes speed up reactions but have no relation to equilibrium or favorability
-The activation energy: energy difference between the reactants and the transition state
-The rate of a reaction is independent of its spontaneity

-For some reactions we go through different intermediates. Which one of them is exactly the one needed and we call it the activation energy (which leads to the transition state of the molecule)?
The one with the highest energy

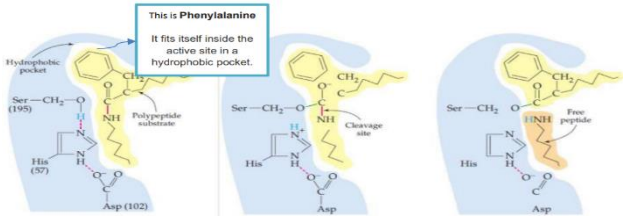


RECALL:
Random Collision Theory: molecules keep Moving in any solution till they find each other → Collision → Energy enough to break the energy barrier

***From a Mechanism Point of View:**

◊ **Proximity Effect:** bring substrate(s) and catalytic sites together, but with no random movement, by having an affinity of these molecules toward the active site

◊ **Orientation Effect:** hold substrate(s) at the exact distance and in the exact orientation necessary for the reaction



◊ **Catalytic Effect:** provide acidic, basic, or other types of groups required for catalysis

◊ **Energy Effect:** lower the energy barrier by inducing strain in bonds in the substrate molecule

STRATEGIES OF CATALYSIS:

***Catalysis by Proximity and Orientation:**

Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis. Such arrangements have been termed **near-attack conformations** (**NACs**); the active site puts the substrate in the nearest attack conformation.

NACs are <u>precursors</u> to reaction transition states

***Catalysis by Bond Strain:**

The induced structural rearrangements produce **strained substrate bonds** reducing the activation energy.

This is a strategy applied by the enzyme **lysozyme** where 3D shape of the monosaccharide will be distorted from the typical **'chair' hexose ring** into the **'sofa' conformation**.

HOW?

1- The active site will enclose the substrate

2- Apply a strain on the bonds to change the angles

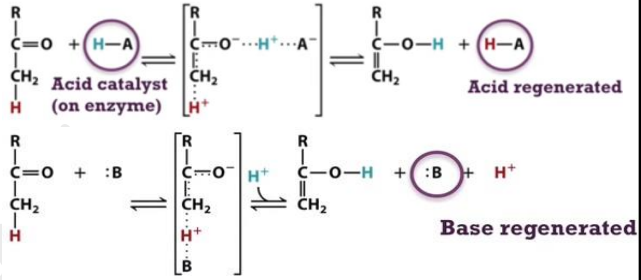
3- Increasing the energy within them

4- Then it can break after

***Catalysis Involving Proton Donors (Acids) and Acceptors (Bases):**

The R groups act as donors or acceptors of protons. **Histidine** is an excellent proton donor/acceptor at physiological pH.

Example: serine proteases



HOW?

1- If you take a proton from the substrate, it will be unstable

2- It will try to abstract a proton from elsewhere

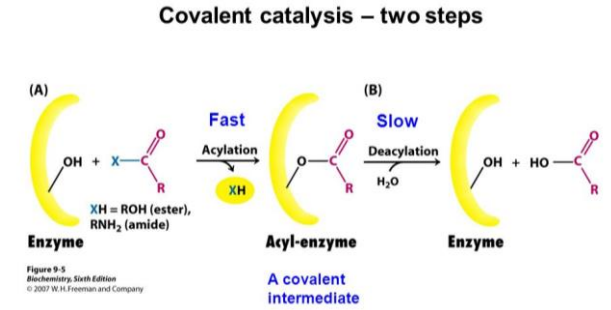
3- The bond will be broken

*** Ionic Catalysis:**

-is the same as what we said about the **acid base catalysis**-

***Covalent Catalysis:**

A lot of enzymes are produced in an inactive manner and then they will be active through covalent catalysis, **by** breaking down of certain piece of the enzyme, that closes the active site



NOTE:
No initial covalent interaction between the active site and the substrate. A covalent bond might occur during the mechanism though, but it'll be broken again
-Covalent Intermediate: forms between the enzyme or coenzyme and the substrate
Example: proteolysis by serine proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase)
[Enzymes preceded by Pro- or followed by -gen adopt this mechanism, i.e. Pepsinogen will be pepsin, Trypsinogen will be trypsin, prothrombin will be thrombin, Fibrinogen will be fibrin**]**

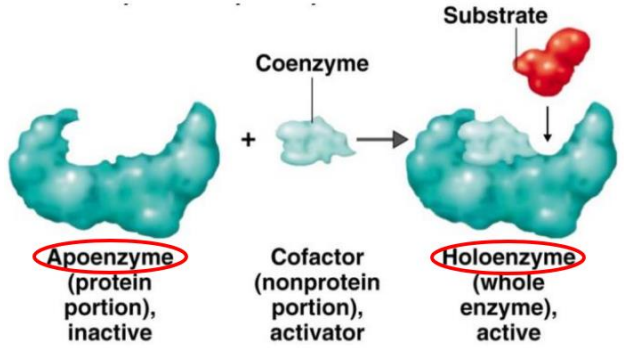
NAMING ENZYMES:
***General View of Naming:**
-In general, enzymes end with the suffix (-ase)
-Most enzymes are named for their **substrates** and for the **type of reactions they catalyze,** with the suffix **-ase** added
i.e. serine hydroxymethyl transferase
-Few enzymes are named for their **products** + synthase
i.e. citrate synthase

NOTE:
ATPase (breaks down ATP) and **ATP synthase** (produces ATP) are the same molecule, its name differs according to the situation

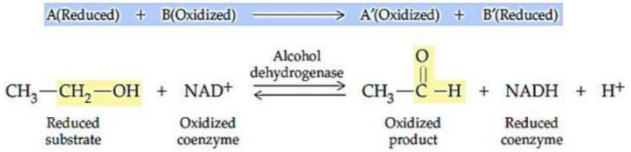
***EC Numbering (Enzyme Commission Numbering):**
-Most scientific method
-EC numbers do not specify enzymes, but enzyme-catalyzed reactions
-Numbering Format:
EC [Major class (1-7)].[Minor class].[Subclass].[further sub-classification]
Example:
EC3: hydrolases
EC3.4: hydrolases that act on peptide bonds
EC3.4.11: hydrolases that cleave off the amino terminal of the amino acid polypeptide
EC3.4.11.4: cleave off the amino-terminal end from a tripeptide

Thus; EC3.4.11.4 is a tripeptide amino peptidase

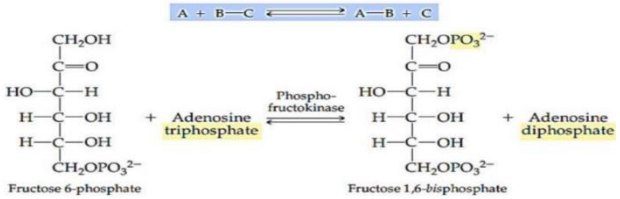
CLASSIFICATION OF ENZYMES:
***According to Structure:**
-Simple; can do its action through the amino acids sequence alone such as trypsin, pepsin
-Complex (conjugated); should have a coenzyme, a cofactor or a metal (non proteineous compound), **i.e.**
Catalase; uses a heme group to break hydrogen peroxide into water and oxygen
RECALL:



***According to Function:**
[Major Classes from 1 to 7]
1) Oxidoreductases: addition or removal of O, O_2, H and require coenzymes (heme)
Reduction: gain of e^- **Oxidation:** loss of e^-
• They occur simultaneously, any oxidation should be accompanied by reduction



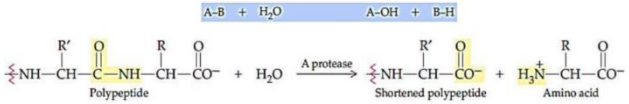
2) Transferases: transfer of a group from one molecule to another, so it should be at least 2 reactants and 2 products in the reaction



3) Hydrolases: addition of water to cleave a covalent bond

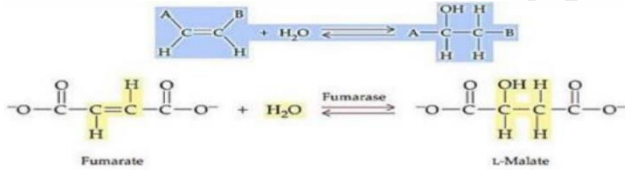
RECALL:

Hydrolysis means using water to breakdown molecules which are made by release of water molecules which involve all macromolecules (lipids, carbohydrates, nucleic acids and proteins)



4) Lyases: addition of a molecule (H_2O, CO_2, NH_3 to a double bond or reverse. (non-hydrolytic).

catalyze the addition or removal of functional groups, but the reaction is coupled to formation or breakdown of a double bond

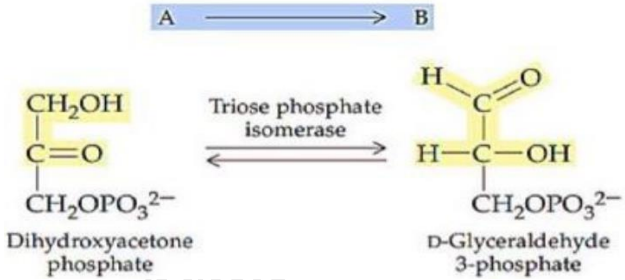


NOTE: Hydrolase vs. Lyase

Lyases can add water as **hydrolases**, but this reaction involves the breakdown of double bond not the whole molecule

In the case of Lyases, one carbon of the double bond takes the OH group and the other takes hydrogen atom, while **hydrolases** break down the whole molecule

5) Isomerases: cause isomeration reaction, with one substrate and one product



NOTE:

Mutases are a subtype of isomerases

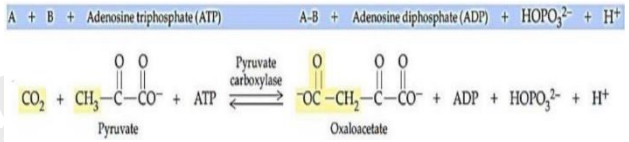
6) Ligases: usually not favorable, so they require a simultaneous hydrolysis reaction.

They build up molecules to make bigger molecules. **Accordingly, those reactions are not favorable**

➢ **The breakdown of a molecule >>** releases energy

➢ **Building up of a molecule >>** requires energy

With these reactions, we can find ATP (not as a source of phosphate only, also as a source of energy)



NOTE:

Transferase gives energy and at the same time it gives the phosphate to bind the substrate of the reaction

In **Ligases**, ATP serves as energy source for the reaction

7) Translocases: Catalyze the movement of ions or molecules across membranes or their separation within membranes, **i.e.** ATP/ADP translocase

- They are enzymes within the membranes mainly and cause the translocation of material from outside to inside or from inside to outside or in both directions
- They also can catalyze the breakdown of a substrate inside the enzyme

Example:

ATP/ADP translocase that translocate ATP from mitochondria (the factory of ATP) to the cytosol to build up molecules and translocate ADP to inside the mitochondria

ENZYMES' MAJOR CLASSES; A CLOSER LOOK:

***Oxidoreductases:**

This group can be further divided into 4 main classes:

1) Dehydrogenases
2) Oxidases
3) Peroxidases
4) Oxygenases

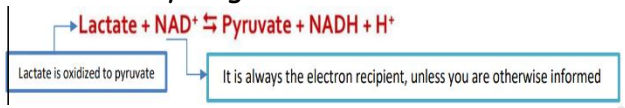
1) Dehydrogenases: catalyze hydrogen transfer from the substrate to a molecule known as **nicotinamide adenine dinucleotide (NAD+)** [most common electron recipient]

→ **They** remove hydrogen, so they oxidize the substrate they have, and they will upload their electrons on electron acceptor (recipient)

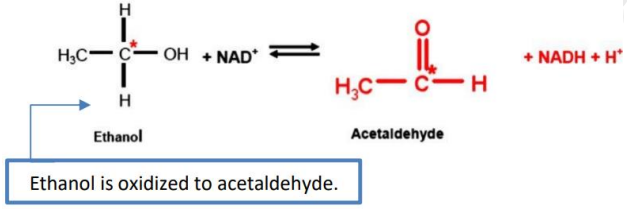
✓ The substrate will be oxidized and NAD+ will be reduced

i.e.

Lactate dehydrogenase



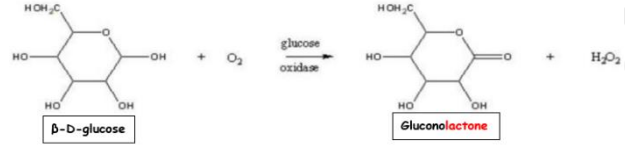
Alcohol dehydrogenase



2) Oxidases: catalyze hydrogen transfer from the substrate to molecular oxygen producing <u>hydrogen peroxide</u> as a by-product [they oxidize molecules using molecular oxygen (O_2)]

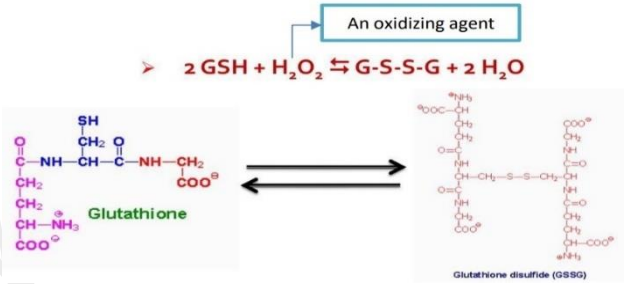
i.e.

Glucose oxidase



3) Peroxidases: catalyze oxidation of a substrate by hydrogen peroxide

i.e. Oxidation of two molecules of <u>glutathione (GSH)</u> in the presence of <u>hydrogen peroxide</u>



4) Oxygenases: behave like **oxidases**, but the difference is that oxygen will not be coupled to hydrogen and get out in the product as a hydrogen peroxide. <u>Oxygen is introduced inside the structure of the substrate</u> [Oxygenases catalyze substrate oxidation by molecular O_2]

- The reduced product of the reaction in this case is **water** and <u>not hydrogen peroxide</u>
- There are two types of **Oxygenases**:

-Monooxygenases: introduce one oxygen atom to the substrate and reduce the other oxygen atom to water (Introducing one of the oxygens inside the substrate)

-Dioxygenases: introduce both atoms of molecular oxygen into the product(s) of the reaction (Introducing both oxygens inside the substrate)

NOTE:

both enzymes are used for synthesis and degradation of heme in our bodies

i.e. Heme oxygenase (a dioxygenase)

***Transferases:**

These enzymes transfer a functional group (C, N, P or S) from one substrate to an acceptor molecule

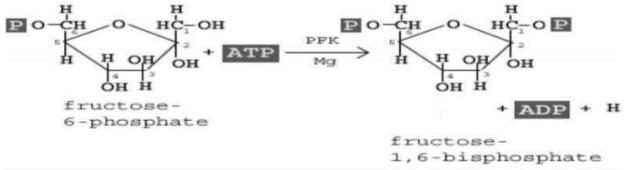
It has two subclasses:

1) Kinases: transfer of a phosphate group from one molecule to another

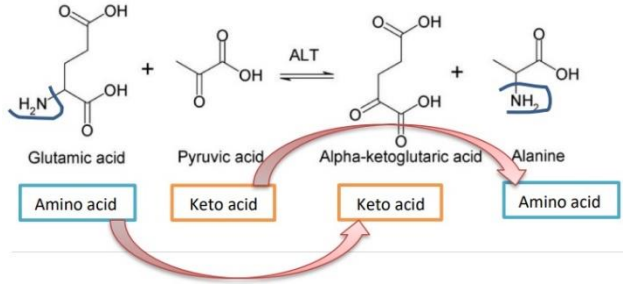
NOTE:

The most common source for phosphate group is **(ATP)**

i.e. Phosphofructokinase
catalyzes transfer of phosphate from ATP to fructose-6 phosphate

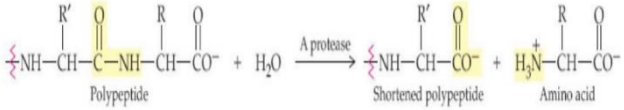


2) Transaminases (Aminotransferases):
transfers an amino functional group from one amino acid to a keto acid (it has an acidic part and ketone part), converting the amino acid to a keto acid and the keto acid to an amino acid
This allows for the interconversion of certain amino acids



Amino Acid	Corresponding Keto Acid
Alanine	Pyruvate
Glutamic Acid (Glutamate)	a-ketoglutaric acid (a-keto glutarate)
Aspartic Acid (Aspartate)	Oxaloacetate

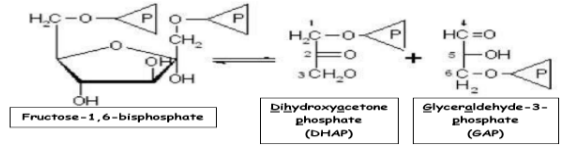
3) Hydrolases:
catalyze cleavage reactions using water across the bond being broken
Examples: Peptidases (Proteases), Esterase, Lipases, Glycosidases, Phosphatases and Nucleases, **named depending on** <u>the type of bond cleaved</u>
i.e. Peptidases (Proteases)
catalyze proteolysis, the hydrolysis of a peptide bond within proteins



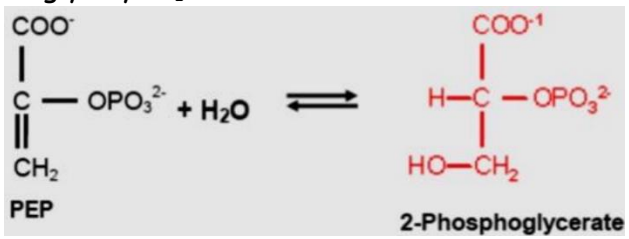
NOTE:
Proteolytic enzymes differ in their degree of substrate Specificity
➢ **Trypsin**; is quite specific; catalyzes the splitting of peptide bonds only on the carboxyl side of <u>lysine and arginine</u>
➢ **Thrombin**; catalyzes the hydrolysis of <u>Arg-Gly bonds</u> in particular peptide sequences only

4) Lyases:
catalyze the addition or removal of functional groups from their substrates with the associated formation or removal of double bonds between C-C, C-O and C-N

i.e. Aldolase

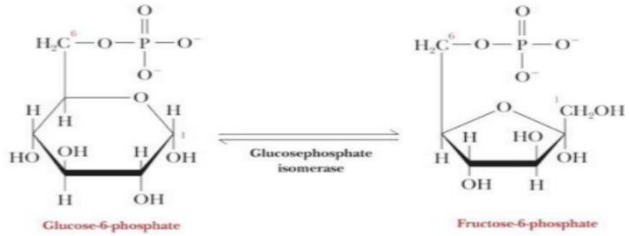


Enolase
interconverts phosphoenolpyruvate (PEP) and 2-phosphoglycerate by formation and removal of double bonds
[The enzyme before the last in the process of glycolysis]



NOTE:
Decarboxylases: a subclass of lyases; it takes out the carboxylic group outside of any substrate releasing CO_2 and forming a double bond

5) Isomerases:
catalyze intermolecular rearrangements
i.e. Glucose-6-phosphate isomerase



RECALL:

Mutases are a sub-type of isomerases

i.e. Phosphoglycerate mutase transfers a phosphate intermediate

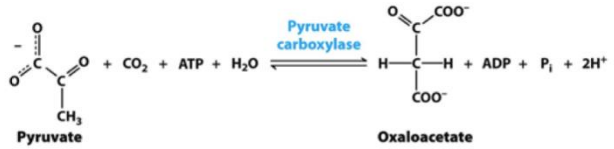
3-P glycerate ⇆ 2 P glycerate

6) Ligases:

join C-C, C-O, C-N, C-S and C-halogen bonds [**ligases are** anabolic enzymes]

The reaction is usually accompanied by the consumption of a high energy compound such as ATP

i.e. Pyruvate carboxylase



You can NOTE that whenever you see in any chemical reaction a CO_2 gets in, it will be a carboxylic group. Whenever you see CO_2 leaving the reaction, it was in the molecule as a carboxylic group

احنا حكينا إنو في عدّة طرق لعمل الإنزيم، و بعد هيك حكينا إنو معظم الإنزيمات بتشتغل لما يكون في مجموعة إضافية، و هسه وضحنا وظائف الإنزيمات .. خلّينا نجمع الأمور مع بعض

RECALL:

-Not all enzymes rely on their active site for catalysis (chymotrypsin vs. conjugated enzymes)

-The side chains of amino acids are polar because there should be **catalysis (binding and losing)**

The hydrophobic interaction causes **binding**, but it doesn't cause **catalysis**

-Things that make enzymes work:

• Functional Groups on Amino Acid Side Chains:

Almost all polar amino acids (nucleophilic catalysis = covalent catalysis), specifically;

Ser, Cys, Lys, & His can participate in covalent catalysis

[Histidine: pKa, physiological pH & *acid-base catalysis***]**

• Coenzymes in Catalysis:

Usually (but not always) synthesized from vitamins

Each coenzyme is specific for a type of reaction. **They are either:**

Activation-transfer coenzymes

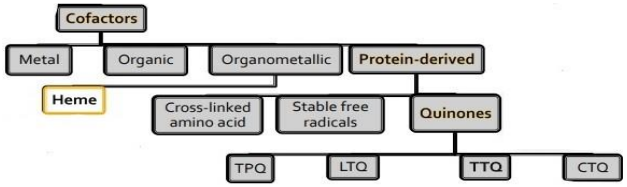
Oxidation-reduction coenzymes

-Enzymes are Conjugated to coenzymes, metal ions or metallocoenzymes

CATALYTIC COENZYMES:

If they are tightly bound to the enzyme and if separated from it, the structure of the enzyme will be degraded or denatured we call them **(prosthetic groups)**

If they are loosely bound to the enzyme, come and go while the structure of the enzyme is preserved, we call them **(co-substrates)**



→ Features of Coenzymes:

- Common to so many reactions
- The original form is regenerated by subsequent reactions
- Synthesized from vitamins
- The amount in the cell is nearly constant

***Activation Transfer Coenzymes:**

Usually participate directly in catalysis by forming a covalent bond

Two groups in the coenzyme:

→ Forms a covalent bond (functional group)

→ Binds tightly to the enzyme (binding group)

NOTE:

Dependence on the enzyme for additional specificity of substrate and additional catalytic power

We have 13 vitamins

4 are fat soluble (A, D, E, K); they don't work as coenzyme

9 are water soluble; all work as coenzyme (they don't work per se (by themselves) except for **vitamin c**, enter as is and work as coenzyme)

The other 8 vitamins are B's vitamins (B1, B2, B3, B5, B6, B7, B9, B12). These 8 vitamins are get modified bound to other structure inside the body, then we name it as a coenzyme, and it helps enzyme to do their work

→ Features of Vitamins:

- They are essential; we cannot synthesis them within our body (there is no pathway in the body to synthesize them)
- We need them to help enzyme in doing their work
- Enzymes are already synthesized in very small amounts so coenzyme and vitamin should be in small amount
- They are organic in their nature

[Lack of vitamins→ Lack of coenzymes→ Lack of the function of the enzymes]

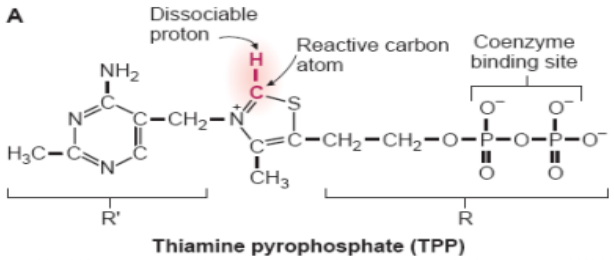
[✓ This definition is species dependent (for one of the species it is vitamin, but it is not for another one)]

NOTE:

Vitamin C, scientifically, you should name it **ascorbic acid**. You cannot name it vitamin c except for humans; for human you can name it either ascorbic acid or vitamin C.

Dogs can synthesize **ascorbic acid**, but it's not a vitamin.

◊ Thiamine pyrophosphate (TPP):



Thiamine pyrophosphate (TPP)

-Source: thiamine (B1)

-Used in decarboxylation reactions

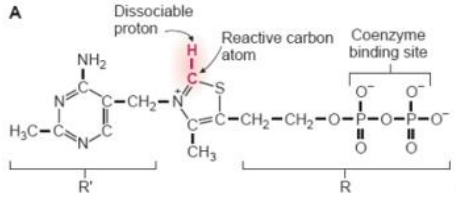
-Reactive thiamine carbon forms a covalent bond with a substrate keto group [binding] while cleaving the adjacent carbon– carbon bond [catalysis]

-Pyrophosphate:

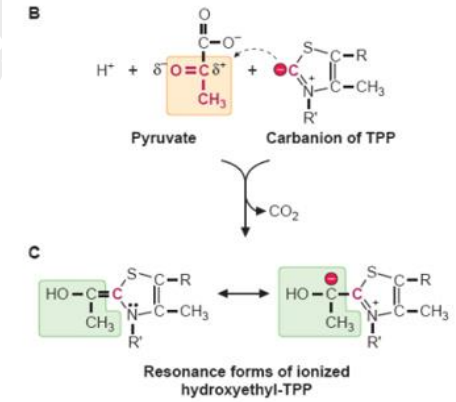
- Provides negatively charged oxygen atoms
- Chelate Mg^{2+} (tight binding)

-Functional group = reactive carbon atom

-Binding group = pyrophosphate



Thiamine pyrophosphate (TPP)



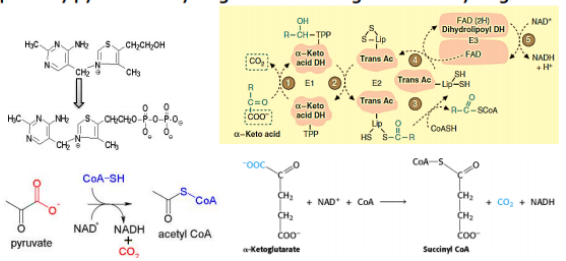
1- H will dissociate from the reactive carbon
2- The carbon will be reactive
3- It will hit the carbon in the substrate
4- The double bond will be lost
5- The bond which connect the carboxyl group will be weak causing the loss of the carboxylic group from the substrate
6- There will be reformation of the double bond

NOTE:

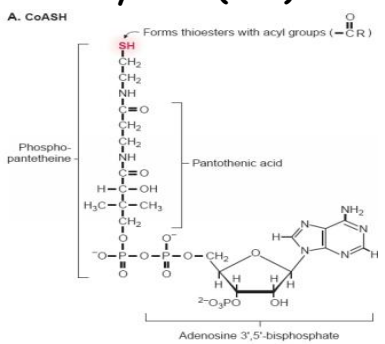
Mg^{2+} or Ca^{2+} are found too; to bind with the negative charges in the phosphates of that coenzyme, which mean binding the enzyme with the coenzyme

Thiamin (vitamin B1)

- Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver
- Required by pyruvate dehydrogenase & α-ketoglutarate dehydrogenase



◊ Coenzyme A (CoA):



Adenosine + Pyrophosphate + Pantothenic acid + modified cysteine (This is why it has SH)

-Source: pantothenate (B5)

-Used in attacking carbonyl groups and forming acyl thioesters

-Adenosine and the Pantothenic Acid:

- Provides negative charges

-Functional group = thiol group (sulfhydryl group)

-Binding group = Adenosine and the Pantothenic Acid

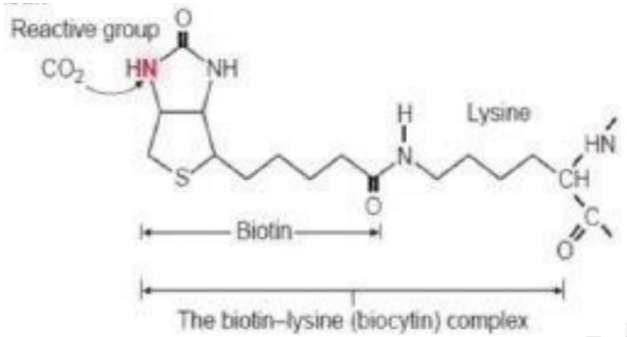
We need CoA as an acyl carrier group.

So, after the breaking down of the peripheral carbon and the carbons are reactive, CoA comes and binds to peripheral carbon causes the stability and then transfer it to another place releases that molecule again.

i.e. Acetyl CoA and Malonyl CoA

◊ Biotin (B7):

Biotin comes as a coenzyme when it gets to the active site of the enzyme, it connects itself *covalently* to the side chain of **Lysine** amino acid, and the whole <u>complex between biotin and lysine</u> is called **Biocytin**



-Obtained from food and intestinal bacteria

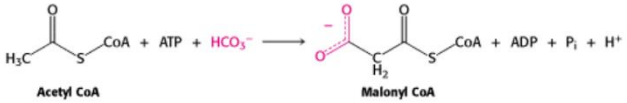
-Used in carboxylation reactions

i.e.

<u>Pyruvate carboxylase</u>

$$\text{Pyruvate} + CO_2 + ATP + H_2O \rightleftharpoons \text{oxaloacetate} + ADP + P_i + 2\,H^+$$

Acetyl CoA carboxylase (fatty acid synthesis)



-Deficiencies are generally seen in:

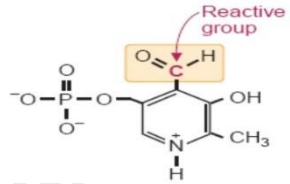
- Long antibiotic therapies

[Biotin is synthesized by the intestinal bacteria**]**

- Excessive consumption of raw eggs (egg white protein, avidin, high affinity for biotin)

[Avidin binds **biotin** and it will get outside of the body without being absorbed**]**

◊ Pyridoxal Phosphate (PLP):



-Source: Pyridoxine (B6)

-Used in the metabolism of amino acids (transaminases)

NOTE: These are reversible reactions

- Mechanism:
  - Reactive aldehyde forms a covalent bond with the amino groups
  - Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)

i.e.

$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

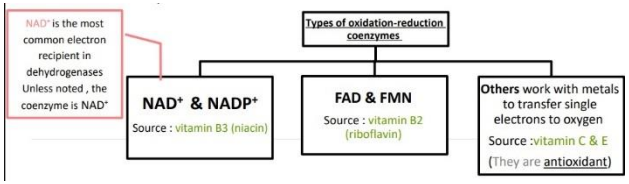
***Oxidation-Reduction Coenzymes:**

Molecules which have the ability of binding to and carrying electrons from one place to another.

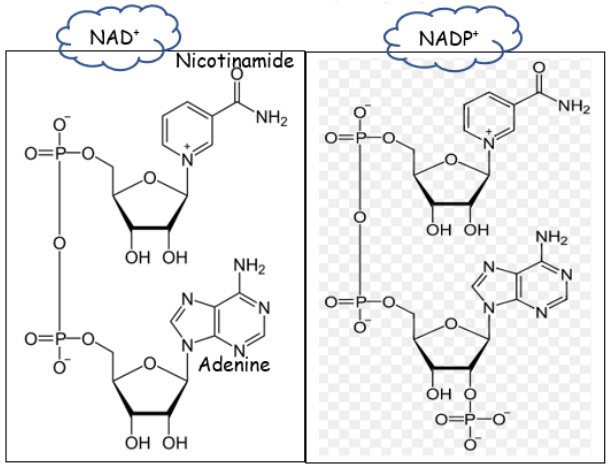
Unlike **activation-transfer coenzymes**, they don't form a covalent bond with substrates; instead, they abstract electrons from them. These coenzymes help **dehydrogenases** function, which are a sub-class of **oxidoreductases** that oxidize a substrate by reducing an electron acceptor, usually $NAD^+/NADP^+$ or a flavin coenzyme such as FAD or FMN.

Dehydrogenases are either:

- Alcohol dehydrogenases; catalyze the interconversion between primary and secondary alcohols to the corresponding aldehyde or ketone
- Lactate Dehydrogenase; catalyzes the interconversion between lactate and pyruvate



◊ NAD^+ and $NADP^+$:



-NAD^+ Found in all living cells

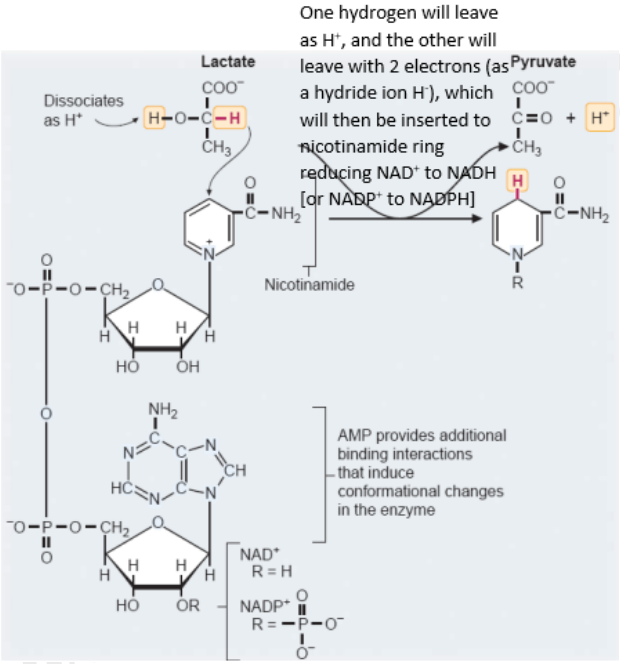
-Why would we have 2 different coenzymes which behave the same?

For regulatory reasons; they are present in different places.

For example, NAD+ is involved in energy metabolism, and NADP+ is involved lipid biosynthesis

-Functional group: C in the nicotinamide ring

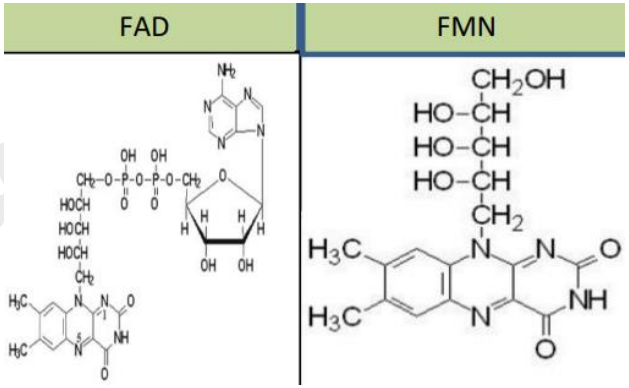
-Binding group: AMP



NOTE:

NAD+ doesn't go into the one electron state [free radical state], which might be cancerous, at all; it either receives the 2 electrons in one step or lose them. So, we find it in solutions swimming freely, **i.e.** mitochondrial matrix, it's not dangerous [the same thing applies to NADP+]

◊ Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN):



-Why would we have 2 different coenzymes which behave the same?

For regulatory reasons and because they differ in space (size)

-Functional group: 2 nitrogen in flavin ring

-Binding group:

NOTE:

The addition of 1 H^+ and 1 e^- occur sequentially in both

FAD → FADH →FADH2

FMN → FMNH → FMNH2

So, it goes into the **free-radical state**, which is dangerous! Because of that, we will never find FAD or FMN in solutions swimming freely; they are hidden inside proteins. FAD and FMN are **prosthetic groups** (tightly bound) to enzymes such as

- succinate dehydrogenase
- pyruvate dehydrogenase complex

NOTE:
Antioxidants are substances that may protect your cells against free radicals, which may play a role in heart disease, cancer and other diseases

WRAP UP:

Water-Soluble Vitamins

Vitamin	Name	Coenzyme or Active Form	Primary biochemical function	
B1	Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer	Helps in decarboxylation rxn
B2	Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer	HELPS IN REDOX RXNS
B3	Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer	
B5	Pantothenic Acid	Coenzyme A (CoA)	Acyl-group transfer	
B6	Pyridoxine	Pyridoxal Phosphate	Amino-group transfer	
B7	Biotin	Biocytin	Carboxyl transfer	
B9	Folate	Tetrahydrofolate	One-Carbon group transfer	
B12	Vitamin B_{12}	Coenzyme B_{12}	1,2 shift hydrogen atoms	
	Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer	TO BE DISCUSSED LATER
C	Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation	

CATALYTIC METALS:
If they are tightly bound to the enzyme and if separated from it, the structure of the enzyme will be degraded or denatured we call them **(metalloenzymes)**
[contribute either to the structure or the catalytic mechanism]
If they are loosely bound to the enzyme, come and go while the structure of the enzyme is preserved, we call them **(metal-activated enzymes)** acting as electrophiles
[the metal is either required or enhances activity (Mg^{2+}, Mn^{2+}, Ca^{2+} and K^{+})]

Examples of metals and their associated enzymes:

Enzyme	Associated Metal
Carbonic anhydrase	Zn^{2+}
Carboxypeptidase	Zn^{2+}
Hexokinase	Mg^{2+}
Glutathione peroxidase	Se^{2+}
Superoxide dismutase	Mn^{2+}

RECALL:
Metals can benefit enzymes by stabilizing the oxyanion which is formed during the catalytic process
***Examples of metalloenzymes [with Zinc as the catalytic metal]:**
◊ **Liver Alcohol Dehydrogenase (ADH);** a dimer, metalloenzyme which have 2 Zn^{2+} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic
It converts ethanol to acetaldehyde
◊ **Carbonic Anhydrase;** zinc atom is essentially always bound to four or more groups

KINETICS OF ENZYMATIC REACTIONS:
***Biochemical Kinetics:** the science that studies rates of chemical reactions what happens throughout the reaction the intermediates, activation energy, etc.
For the rxn: A → P

or

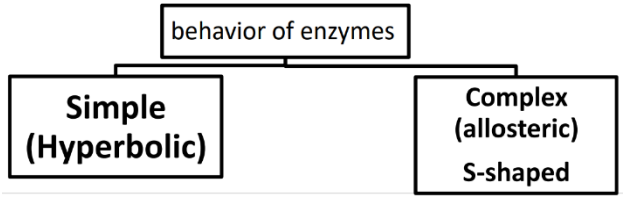
$$v = \frac{d[P]}{dt} \qquad v = \frac{-d[A]}{dt}$$

NOTE:
The negative sign means the substance is consumed
For the rxn: A + B → P

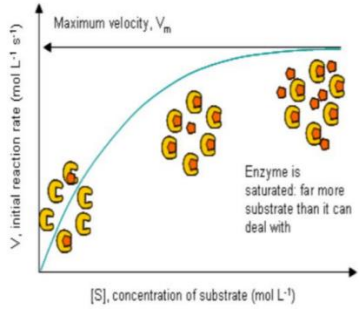
$$\text{Rate} = \frac{-\Delta[A]}{\Delta t} = \frac{-\Delta[B]}{\Delta t} = \frac{\Delta[P]}{\Delta t} \qquad v = \frac{-d[A]}{dt} = k[A]$$

The rate is proportional to the concentration of A, and k is the rate constant
RECALL:
Rate (v) = $K \times [A]^y \times [B]^z$
Overall order = y + z

***Michaelis and Menten Studied Enzymes' Behaviors:**



◊ **Simple (Hyperbolic) Curve [Behavior]:**



-Maximal rate (V_{max}):

- the number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated by the substrate
- is achieved when the catalytic sites on the enzyme are saturated with substrate
- reveals the **turnover number** of an enzyme
- At Vmax, the reaction is in *zero-order rate* since the substrate has no influence on the rate of the reaction
- Each enzyme has a specific Vmax with respect with substrate it deals with

-Michaelis-Menten Equation:

NOTE:

Derived from the *Steady State Assumption*

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$K_m = \frac{\text{rates of degredation of ES comlex}}{\textit{rates of formation of ES complex}}$$

$$k_m = \frac{k_{-1}+k_2}{k_1}$$

-This equation is based on:

- The production of the product is irreversible, the reaction goes in one way, accordingly there is no K-2
- The Steady State Assumption, the formation and degradation of the enzyme-substrate complex (ES complex) are in the same rate, ES is in a steady state

NOTE:

K_m describes the affinity of enzyme for the substrate; the smaller the Km, the greater the affinity and vice versa. Furthermore, the dissociation constant **K_D** (= k-1 /k1) is the actual measure of the affinity

[K_m gives us a LOWER affinity when compared to **K_d**, but **K_m** is less expensive to be done**]**

Solving Equation;

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$V = \frac{k_{cat}[E_0][S]}{K_m + [S]}$$

- K_m is the Concentration of substrate needed to reach the V_0=1/2 V_{max}
- K_m is dissociation constant over association constants

WRAPPING UP CONCEPTS:

-If we increase the enzyme concentration,

- V_{max} will increase
- K_m is constant

-Comparing the efficiency of different enzymes is based on equal concentrations

-Turnover number (K_{cat}); the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated

[K_{cat} = $V_{max}/[E]_T$**]**

→ **Time for a single reaction** = $1/K_{cat}$

-Reaction rate (Velocity); measures the *concentration* of substrate consumed [negative] (or product produced [positive]) per unit time (mol/(L.s) or M/s)

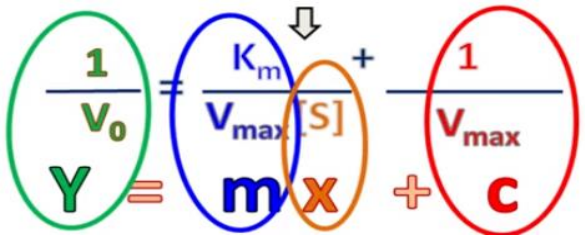
-Enzyme activity; measures the *number of moles* of substrate consumed (or product produced) per unit time (mol/s)

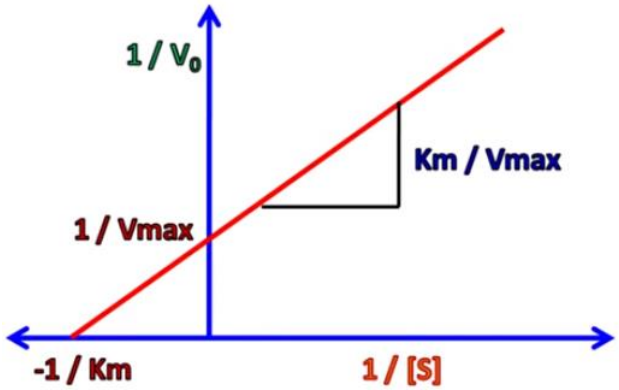
Enzyme activity = rate of reaction × reaction volume

-**Specific activity**; measures moles of substrate converted per unit time per unit mass of enzyme (mol/(s.g))
[used in comparison between different enzymes in different masses. This is useful in determining enzyme purity after purification**]**
Specific activity = enzyme activity / actual mass of enzyme
-**Turnover number**; measures moles of substrate converted per unit time per moles of enzyme (min^{-1} or s^{-1})
Turnover number = specific activity × molecular weight of enzyme

***Problem Raised; Disadvantages of Michaels-Menten Equation:**
• We need to use a huge amount (excess) of substrate in order to reach the Vmax, which needs a lot of experiments, power, time and money
• Inaccuracy of K_m and V_{max} values

***Problem Solved; Lineweaver-Burk Plot (Double Reciprocal Plot):**





ENZYME REGULATION:
***Specific Regulation:**
-Isozymes
-Inhibition
-Conformation
-Amount
-None- specifically

◊ Isoenzymes:
Enzymes with different sequences of amino acids, different structures, but all catalyze the same reaction, each one in a different tissue in the body, same substrate and product, but different gene, different localization, different parameters (K_m, V_{max}, K_{cat})

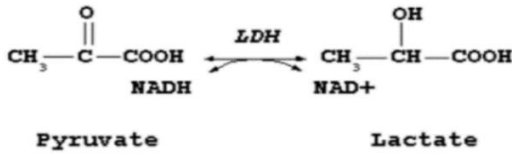
Example 1: Hexokinase
-Responsible for phosphorylating glucose; thus, trapping it
-There are two isozymes for glucokinase:
• Hexokinase I (RBCs)
• Hexokinase IV (glucokinase, liver, pancreas)
-What are the differences between the RBCs and the liver cells?
• **RBCs** don't have mitochondria; thus, they depend on glycolysis (in cytosol) for energy production (few energy) so it needs glucose to exist permanently inside the cells, however, **liver cells** have mitochondria as a result they produce good amounts of energy
• **RBCs** only consume glucose by converting it to energy by glycolysis, however, **liver cells** consume and also stores glucose as glycogen

-Thus, what are the differences between Hexokinase I (RBCs) and Hexokinase IV (liver cells)?
• K_m values:
for glucose in hexokinase I = .1 mM; higher affinity
for glucose in hexokinase IV = 10 mM; lower affinity
K_m < [S] = Effective binding
K_m > [S] = Ineffective binding

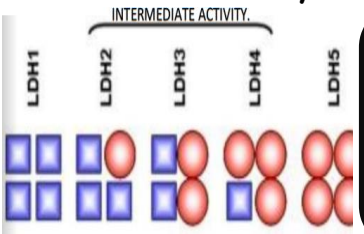
• Feedback Inhibition:
Hexokinase I will be inhibited at a certain point
Hexokinase IV won't be inhibited;
in liver cells; no glucose-6P enough to cause feedback inhibition, because of the conversion of glucose into glycogen
in pancreatic cells; must always sense the concentration of the glucose in the blood regardless of its concentration; thus, no feedback inhibition

Example 2: Lactate Dehydrogenase (LDH)

-Responsible for the interconversion between lactate and pyruvate



-There are five isozymes for glucokinase:



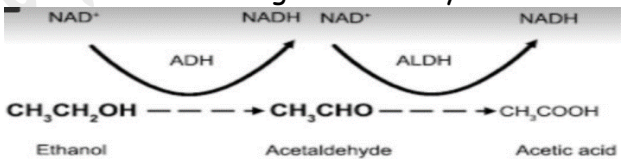
LDH1 (H4) = Heart
LDH5 (M4) = Skeletal muscles

-What are the differences between the heart muscle and skeletal muscles?
Heart muscle mustn't get fatigue due to the accumulation of lactate

-Thus, what are the differences between LDH1 [H4] (heart muscle) and LDH5 [M4] (skeletal muscles)?
• K_m values:
for pyruvate in LDH1 > for pyruvate in LDH5
• Inhibition:
In the heart, if there is a high concentration of pyruvate and the enzyme started binding pyruvate molecules, the active site is designed in a way that after binding the enzyme will be broken "substrate inhibition", so the enzyme won't go in the forward direction, however, when lactate binds to H4 (in the heart), there will be no inhibition and it will be converted into pyruvate
In skeletal muscles, M4 is not inhibited, any pyruvate that binds to the enzyme will be converted to lactate

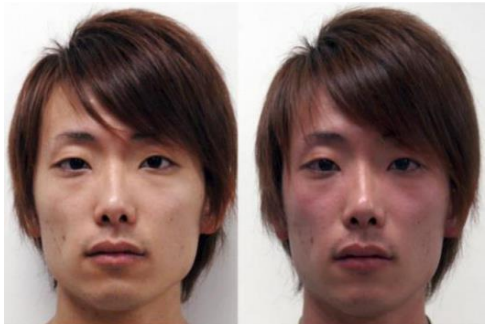
Example 3: Aldehyde Dehydrogenase (ALDH)

-Responsible for oxidizing acetaldehyde to acetate; neutralizing the toxicity of alcohol



-There are four tetrameric isozymes for ALDH, **two in the hepatic (liver) cells:**
• Cytosolic (ALDH II); higher K_m
• Mitochondrial (ALDH I); lower K_m

NOTE:
People who have **mitochondrial (ALDH I)** mutated, have accumulated **acetaldehyde** due to low affinity for it by the enzyme ALDH; thus, leaves the cells and heading to the blood stream therefore the toxicity increases in the blood and symptoms like tachycardia and *flush* response take place, after drinking alcohol
→ 50% of Japanese and Chinese are unable to produce ALDH I (not observed in Caucasian and Negroid populations)



◊ Inhibition:
-Inhibitors are classified into:
→ **reversible;** which are considered physiological, normally take place in our bodies
→ **irreversible;** they are obtained (acquired), our bodies don't synthesize them normally, such as toxins, poisons and drugs

"Irreversible inhibitors"

→ These inhibitors mimic or participate in an intermediate step of the catalytic reaction

→ The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzymes

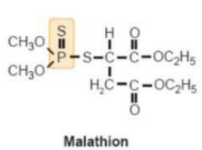
→ Covalently Bounded Inhibitors:

Examples:

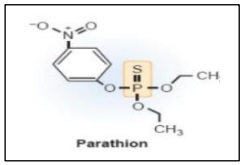
- **The lethal compound (DFP):**

is organophosphorus, as it contains phosphate group that, serves as a prototype for:

→ **Insecticides** such as: malathion, parathion

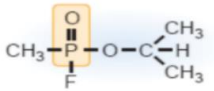


Malathion



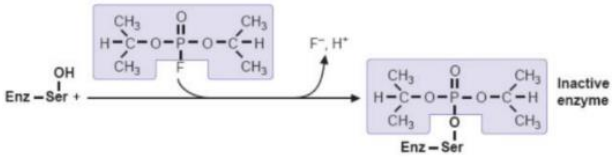
Parathion

→ **Nerve gases** used in wars such as: sarin gas; breathing this gas causes suffocation within a short amount of time



Sarin

Organophosphorus compounds target **acetylcholinesterase**, which is responsible for breaking **acetylcholine** and relax muscles, and bind *covalently* with its active site through **serine**



Tetanus of muscles means that the muscle is unable to work and function as a result of **energy depletion**. The effect on the skeletal muscles is not dangerous, however, the danger is in the energy depletion of the diaphragm, this will cause respiratory arrest, and the patient will die

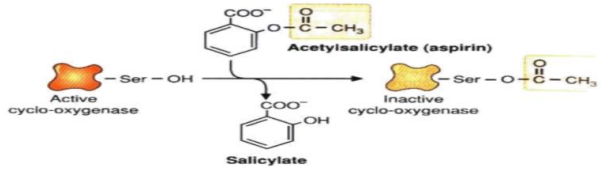
NOTE:

DFP also inhibits other enzymes that use serine, **i.e.** serine protease, but the inhibition is not as lethal

---

- **Aspirin (Acetylsalicylic Acid):**

Aspirin targets **cyclo-oxygenase (prostaglandin endoperoxide synthase)**, which is responsible for converting **arachidonic fatty acid**, into different prostaglandins, leukotriene, thromboxane, etc. [inflammatory agents], and bind with its active site through **serine**



→ Transition-State Analogs and Compounds that resemble Intermediate

Stages of the reaction:

Substrate analogs bind more tightly (with higher affinity) than substrates, but with no reaction taking place (inhibition)

Because the enzyme itself binds the analog, it seems that it chooses to kill itself, therefore these inhibitors are called **suicide inhibitors**

NOTE:

Inhibitors that undergo **partial reaction** to form irreversible inhibition in the active site are sometimes termed suicide inhibitors

Examples:

- **Methotrexate:**

-A synthetic inhibitor

-The most common drug used for breast cancer (anti-cancerous)

Methotrexate targets **dihydrofolate reductase**, which is responsible for converting **dihydrofolate** into **tetrahydrofolate** which is used by **thymidylate synthase** to methylate **uracil** into **thymine**, and **DHFR** binds **MTX** 1000-fold more tightly than **DHF**

Therefore, it inhibits nucleotides synthesis, and DNA, RNA synthesis, and accordingly there will be no new cells and this is how it fights cancer

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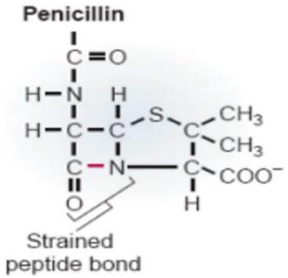
•**Penicillin:**

-An antibiotic



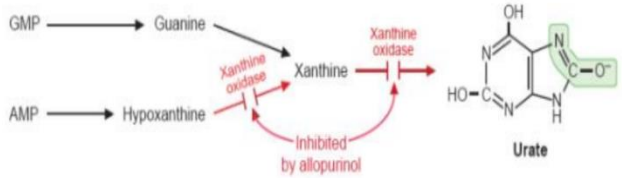
It contains beta lactam ring, which has a nitrogen atom connected to a carbonyl group in an amide bond, which looks like a peptide bond

Glycopeptide transferase or transpeptidase binds **penicillin** with higher affinity than the original substrate, and it will break down the strained peptide bond in penicillin thinking that it is a peptide bond but it is not, so the reaction will not take place, the enzyme will be inhibited and accordingly, the bacterial membrane won't be built, which kills the bacteria

---

•**Allopurinol:**

-A drug used to treat **gout**; a disease that is caused by the accumulation of **urate crystals** (insoluble) in joints mainly in the big toe, which causes inflammation of the joint and friction leading to pain



When we eat food with high content of purines, the amount of xanthine increases, then the oxidation of xanthine by xanthine oxidase increases which will produce **urate crystals**

→ The active site affinity to **allopurinol** is much higher than it is to **hypoxanthine**, therefore the **xanthine oxidase** oxidizes the drug **allopurinol** to **oxypurinol**, which binds very tightly to a *molybdenum-sulfide complex* in the active site; inhibiting the enzyme to function

→ **Heavy Metals:**

Tight binding of a metal to a functional group in an enzyme such as: **Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)**. Metals are relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity. Over normal values of **mercury and lead** causes serious abnormalities, because they bind to the enzymes with a very high affinity compared to the metals that are normally present inside the enzymes

•**Mercury:**

binds to so many enzymes, often at reactive **sulfhydryl groups (cysteine)** in the active site. It binds covalently to the thiol group, in a ligation bond, which is a very strong bond

---

•**Lead:**

-It inhibits through replacing the normal functional metal in an enzyme

-It can be either:

reversible; competing with zinc in the active site of the enzymes that synthesize the heme

irreversible; due to its ability to replace Ca^{2+} in several regulatory proteins (have higher affinity to lead compared to calcium) that are important in the central nervous system and other tissues

NOTE:

If pieces of lead-containing dry painting were eaten by a child, and as his blood brain barrier isn't fully tightened, **lead** will reach his brain and accumulate there leading to an *irreversible retardation*

"Reversible inhibitors"

→ bind enzymes noncovalently [while irreversible inhibitors bind enzymes in a covalent manner]

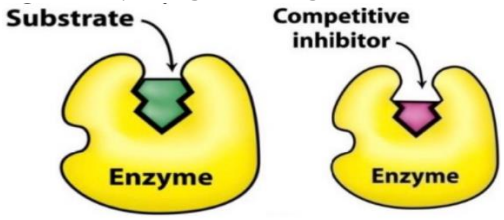
→ dissociate/associate rapidly with enzymes.

What binds the enzyme more (substrate or inhibitor)?

depends on the affinity of the inhibitor and the conc. of the inhibitor in the solution

→ Competitive Inhibition:

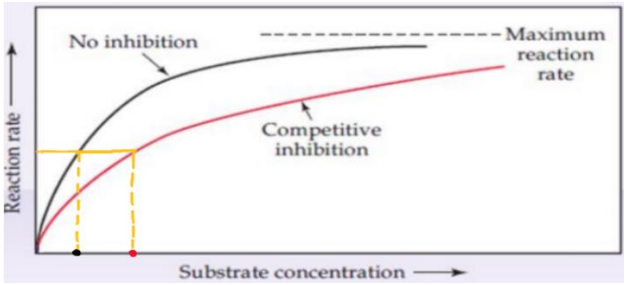
-An inhibitor that settles *inside* the active side <u>competing</u> the original substrate

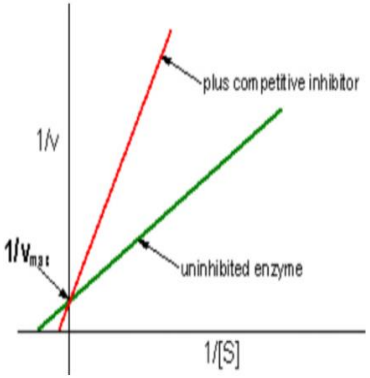


-Which one will bind the active site depends on:

<u>Affinity</u>

<u>Concentration</u>; if we have higher conc. of the substrate it will eliminate the effect of the inhibitor even if the affinity is high for the inhibitor

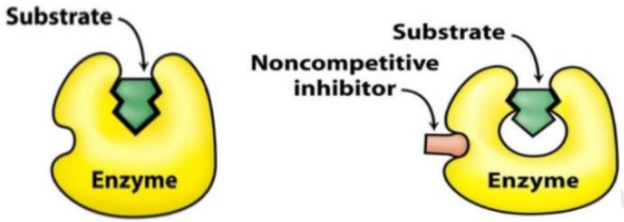


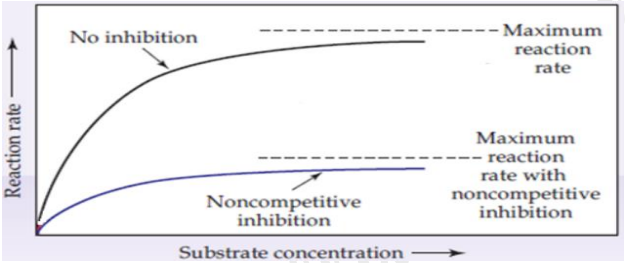


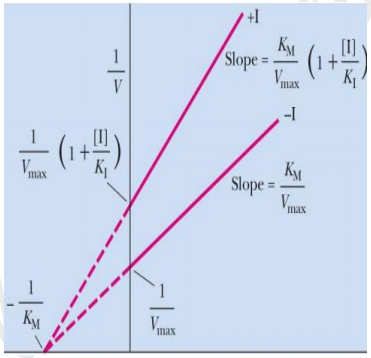
With;
V_{max} is constant
K_m will increase
Slope will increase

→ Competitive Inhibition:

-An inhibitor that settles *outside* the active side <u>competing</u> the original substrate







With;
V_{max} will decrease
K_m is constant
Slope will increase

◊ Conformation:

-Allosteric activation and inhibition

-Phosphorylation or other covalent modification

-Protein-protein interactions between regulatory and catalytic subunits or between two proteins

-Proteolytic cleavage

RECALL:

-Proteins/Enzymes follow either the hyperbolic curve (**i.e.** <u>Chymotrypsin</u>, <u>Myoglobin</u>) or the sigmoidal curve (**i.e.** <u>Aspartate transcarbamoylase (ATCase)</u>, <u>Hemoglobin</u>)

-The sigmoidal curve indicates:

- Cooperativity
- Protein is allosteric

"Allosteric Regulation"

-Allosteric Enzymes: A multi-subunit enzyme with catalytic subunit(s) -with active site(s)- and regulatory subunit(s) -without active sites-; whereas the catalytic subunit can be considered regulatory to the other subunit

-Their Effectors can be either:

- **Homotropic**; same material binds to the active site and regulatory site, **i.e.** <u>O_2 for hemoglobin</u>
- **Heterotropic**; different materials bind to the active site and regulatory site, **i.e.** <u>CO_2 for hemoglobin</u>

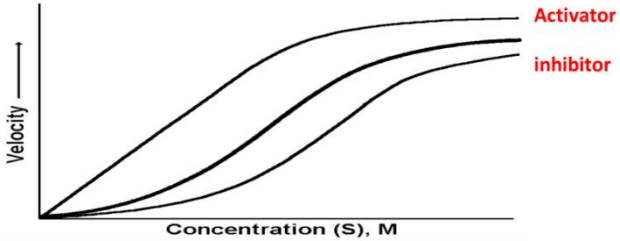
→ Binding triggers a conformational change in the active site;

more efficient = **activator**
less efficient = **inhibitor**

RECALL:
The Michaelis-Menten model can't explain the kinetic properties for allosteric enzymes

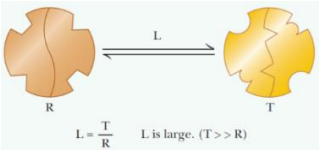
Simple	Allosteric
V_{max}	V_{max}
K_m	$K_{0.5}$

NOTE:
Allosteric inhibitors have a much stronger effect on enzyme velocity

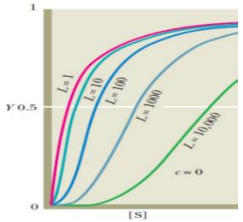


Activator; V_{max} is constant | $K_{0.5}$ is less
Inhibitor; V_{max} is constant | $K_{0.5}$ is less

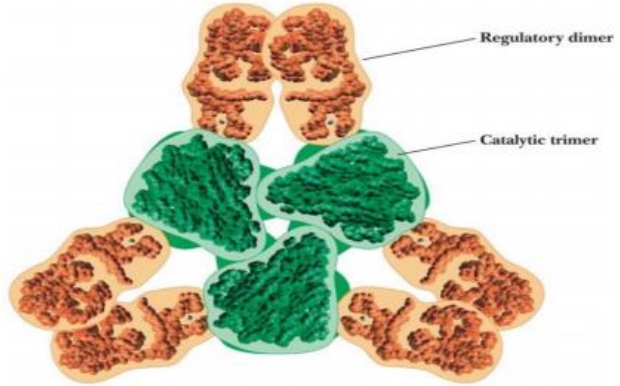
-The Two Conformations of Allosteric Enzymes:
In the presence of activator, we will have more relaxed conformation (R), while in presence of inhibitor, we will have more taut conformation (T)

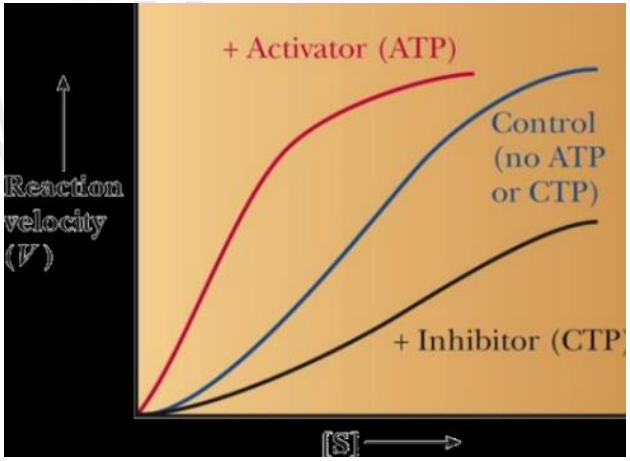


→ As L (T/R) increases, the shape becomes more sigmoidal



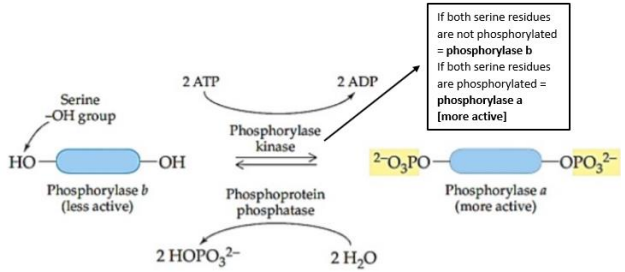
Example: Aspartate transcarbamoylase (ATCase)





"Covalent Modification: Phosphorylation"
-Adds two negative charges; new electrostatic interactions and accordingly conformation
-Can take place in less than a second
-Often causes highly amplified effects
-Reversible
-Can form three or more hydrogen bonds: specific interactions with hydrogen-bond donors
[Phosphate group binds to very specific areas on proteins, it binds hydroxyl group on serine, thyronine, or tyrosine]

Example: Glycogen phosphorylase
-**Ser** is away from the active site
-Mostly, **ATP** is the donor

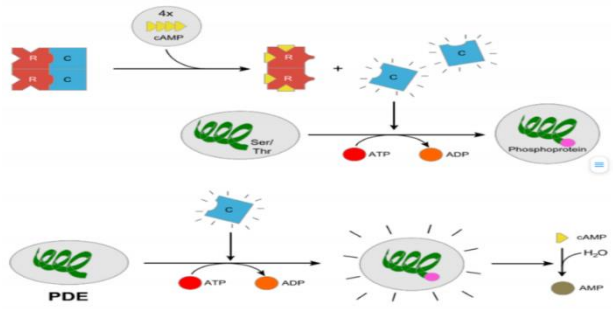


NOTE:
-Weather phosphorylase b, in T or R state, it would be phosphorylated and converted to the active form of phosphorylase a. but in its inactive form (phosphorylase b), there would be more molecules in T state than in R state.

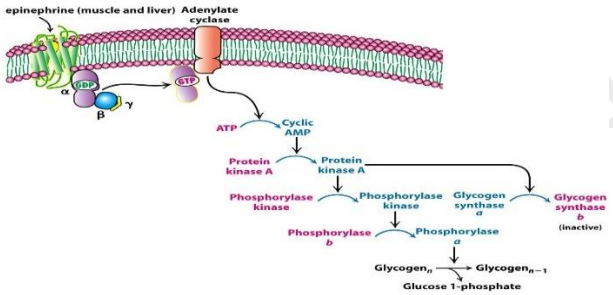
-The transition of phosphorylase between the T and the R state is controlled by the energy charge of the muscle cell.

NOW, What Enzymes Can Phosphorylate Other Enzymes -as in the case above-?
One common phosphorylating enzyme is Protein Kinase A (PKA)

PKA: an enzyme whose activity is dependent on cellular levels of cyclic AMP (cAMP) that phosphorylates many enzymes and protein that is downstream for it, inhibits some of them and activates others, **i.e.** Phosphodiesterase enzyme (PDE).



Glycogen Phosphorylase.



Other Covalent Modifications:

- **Adenylylation process**

addition of adenylyl group). AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes

- **Uridylylation:**

Addition of uridylyl group. It can cause activation or inhibition of a certain enzyme

- **ADB ribosylation:**

ADP ribose and ADP glucose are there in the process to cause activation or inhibition and we call it the high energy intermediate in the pathways

- **Methylation:**

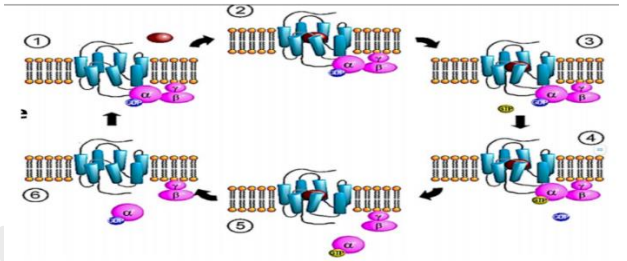
Adding a methyl group. Methyl group has a positive charge. So, this process masks the negative charges accordingly to increase the hydrophobicity on carboxylate side chains

- **Acetylation:**

Adding acetyl groups. The acetyl group has a negative charge

"Protein-Protein Interaction"

i.e. G_α of G-proteins



RAS is an example too

"Proteolytic Cleavage"

NOTE:
This is an *irreversible cut* usually at the N-terminus
i.e. chymotrypsinogen → chymotrypsin
NOTE:
Chymotrypsinogen: single polypeptide chain (245 residues), 5 (S—S) bonds
-This pathway of regulation occurs to enzymes that you do not need it to work in the area where the production is
i.e.
Digestive enzymes; trypsin, chymotrypsin, pepsin
Enzymes related to blood clotting; thrombin, fibrin
[Distinguished by the prefix (Pro-) or the suffix (-gen)**]**

***Non-specific Regulation:**
-Slower than specific regulation
-Control the amount of enzymes

→ Regulated Enzymes:
By increasing or decreasing the rate of gene transcription (induction and repression).
Takes hours to days.

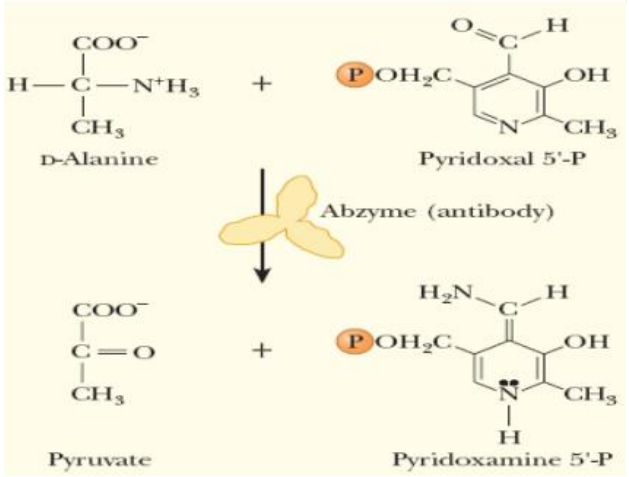
→ Regulated Protein Degradation:
- A characteristic half-life within lysosomes
- During fasting or infective stress: gluconeogenesis and synthesis of antibodies increase
- Increased synthesis of **ubiquitin**

◊ The Effect of Temperature:
Increase in T° increases the rate until reaches a max. Around 40°C many enzymes are denatured. At 50°C most enzymes will be denatured

◊ The Effect of pH:
Changing of pH cause denaturation of enzymes, because it affects the ionic interaction inside the protein
Pepsin; best at low pH
Cholinesterase; best at pH = 7
Chymotrypsin; best at pH = 8

If the 3D structure of an enzyme is not held via ionic interactions, it won't be affected with the change of pH
i.e. papain enzyme

ABZYMES:
-Abzymes are antibodies that have catalytic activity and are produced against a transition state analog



NOTE:
تستخدم هذه الطريقة لمعالجة المدمنين, بحيث يتم حقن المريض بهذه ال abzymes والتي تقوم بتكسير الكوكائين لجزيئين غير ضارين لا يسببوا الإدمان فيقل تركيز الكوكائين في جسم المريض.

RIBOZYMES:
-Ribozymes are not proteins, instead, they are RNA molecules that has properties of catalyzing chemical reactions related to RNA, like: splicing and protein synthesis

NOTE:
The catalytic efficiency of catalytic RNAs is much less than that of protein enzymes, it can be greatly enhanced by binding to specific protein, even though it still less than protein enzymes

REGULATION OF METABOILC PATHWAYS:
***Pathways (Series of Reactions) Can Have Many Types:**

Pathway	Enzymes used in each step	Final Product
Linear	Different	New
Cyclic	Different	1st reactant
Spiral	Same	New

NOTE:
Regulation at the first step is very high yield, while at the last step is low yield
***How to Regulate?**
◊ Counter-regulation of Opposing Pathways:
In our bodies, synthesizing reactions occur in specific place away from degradation reactions.
[All *degradation* reactions occur in the **mitochondria** (except for the glycolysis),

and all *synthesizing* reactions occur in the **cytosol**, so they don't occur in the same place**]**

◊ Tissue Isozymes for Regulatory Proteins:

By putting every isozyme in different places making the reaction behave differently in different tissues

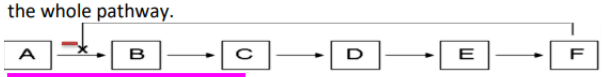
◊ Regulation at the Rate-Limiting Step:

The rate determining step is the slowest step, called **the committed step**, which is the first irreversible step, as well;

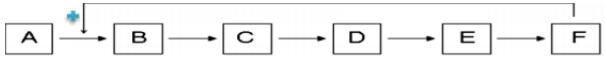
- it requires high amount of energy (has high activation energy)
- it has high K_m values

◊ Feedback Regulation:

Negative feedback regulation: If the final material is increased to a certain level it will come back and inhibits the first enzyme that catalyses the whole pathway.



Positive feedback regulation: If the concentration of the final material increased to a certain level it will come back and give more activation to the enzyme that catalyses the first step (the body use it when it needs more and more of the product).



Feed- forward regulation: when the first intermediate comes out it goes to a step ahead of it and catalyses the enzyme ahead to it to be more active, so whenever the intermediate comes, it will be consumed directly (the body use it when it wants to get red of toxic intermediates in the liver by increasing the prosses of consuming these toxic materials into nontoxic products).

NOTE:

This type of regulation is much slower to respond to changing conditions than allosteric regulation

◊ Enzyme Compartmentalization:

[Enzymes are sequestered inside compartments where access to their substrates is limited**]** = we put the enzymes and their substrates together in a certain part to *fasten the reaction* to occur and *reducing the area of diffusion*, **for example:**

-Hydrolytic enzymes are in the lysosomes

-All the energy metabolic pathways are in the mitochondria

-Synthesis of fatty acids occur in the cytosol, while their degradation occurs in the mitochondria

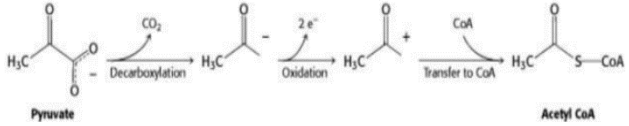
◊ Enzyme Complexing (a Multienzyme Complex):

More than one enzyme connected to each other, in order for the product of each step to continue as a reactant in the following step, without consuming time

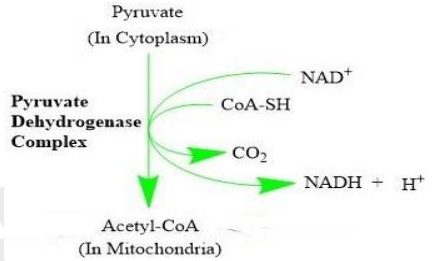
i.e.

Pyruvate Dehydrogenase Complex

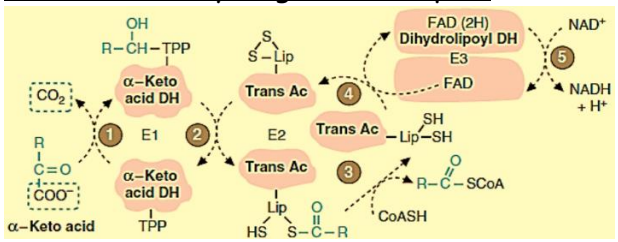
3 steps are:



The complex achieve them with one shot:



α-Ketoacid Dehydrogenase Complex



ENZYMES IN MEDICAL DIAGNOSIS:

***Concept:**

if certain enzymes are found in certain cells, and these cells died and disintegrated, these enzymes will diffuse to the ECM then to the blood, so if we take a blood sample, we will find that these enzymes are present in the blood in higher concentration than normal, and in this way we can know that there's a problem in the cells that contain these enzymes

***Used Enzymes:**

Enzyme	Original Place
ALT	Liver
AST	Liver + Heart

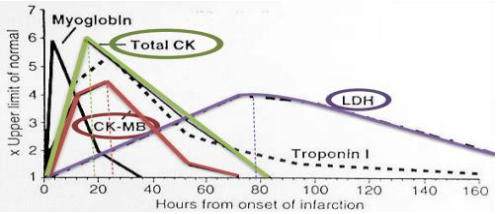
LDH	Heart + Skeletal muscles + Blood
CK (CPK)	Heart + Muscles + Brain

◊ For Liver Diseases:

-Liver disease or damage → ALT/AST < 1

-Liver hepatitis → ALT/AST > 1

◊ For Heart Diseases; Myocardial Infraction (MI):



-Normal → LDH1/LDH2 < 1

-Abnormal (MI) → LDH1/LDH2 > 1

***CK (CPK):**

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

We use BB for brain diagnosis **and** MB for heart diagnosis

NOTE:

بشكل عام يصعب فحص كل نوع على حده, بل يتم فحصها معاً (أي أننا نفحص CPK كاملاً وليس أنواعه الفرعية) ونستطيع التنبؤ بمصدرها من الأعراض عند المريض ومكان الألم (مثلا: ألم في الرأس-مشكلة في الدماغ/ ألم في الصدر-مشكلة في القلب.. وهكذا).

**NOTES:

- Most of released CPK after MI (الجلطة القلبية) is CPK-MB.
- Increased ratio of CPK-MB/total CPK may diagnose acute (حاد)infarction, but an increase of total CPK in itself may not.

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