Lecture : 22 (7th After Mid)

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☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry

biometrics biochemy ecology bionomics taxonomy biophysics bacteriology biological radiobiology anatomy science microbiology life cytology molecular pharmacology astrobiology exobiology gnotobiotics biochemics physiology biology bioecology biometry enzymology bionics genetics ethnobiology

Mousa Suboh

Biochemistry Sheet, lecture #22
Methods for/of Regulating the Enzymatic Function

Content Outline:

Methods for/of regulating enzymatic activity:
1) Isozymes AKA Isoenzymes
2) Inhibition:
   a) Irreversible:
      - Covalent Inhibitors
      - Transition-State Analogs:
      - Heavy Metals:
   b) Reversible:
      - Competitive Inhibition
      - Non-competitive Inhibition
3) Conformational Modification:
   a) Allosteric Enzymes
   b) Covalent Bonding
   c) Protein-protein interactions
   d) Proteolytic cleavage

1) Isozymes or Isoenzymes:

Isoenzymes :enzymes that differ in amino acid sequence but catalyze the same reaction , isoenzymes can regulate enzymatic function by creating different copies of the enzyme, the copies could differ in a small number of amino acids or in the number of subunits (polypeptide chains), for example an enzyme could have 1 subunit and its isozyme could have 2subunits but The molecular weight could be similar or very different. We took 3 Examples:

- Hexokinases
- Lactate dehydrogenases
- Aldehyde dehydrogenases

2) Inhibition:-

They are classified into 2 types: a) irreversible b) reversible.

a) Irreversible Inhibition:

Also known as Mechanism-Based Inhibition, because the inhibitors inhibit enzymes through their Mechanism they are classified into :-

- Covalent Inhibitors: (2Examples)

Example1: DFP

The inhibitor is DFP and many compounds can be synthesized from it like :-
1) nerve gas (sarin)- 2) insecticides مبيدات حشرية like Malathion and Parathion.

The effect of DFP could be either lethal or not it can be Lethal: when it inhibits or attacks the enzyme acetylcholine esterase it can be Not lethal: when it inhibits or attacks serine proteases.

Example2: Aspirin

Aspirin, it irreversibly inhibits the enzyme cyclooxygenase and inactivates its function.

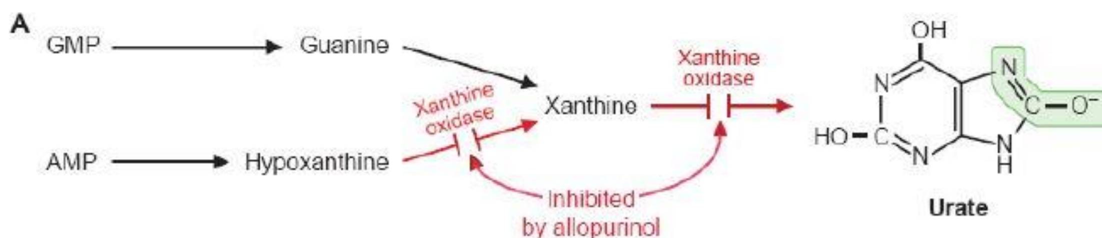
- Transition-State Analog Inhibitors:

they are chemical compounds that resemble the transition state of a substrate in an enzyme catalyzed reaction.

we can't synthesis molecules that precisely mimic the transitional state itself or synthesize a material that is 100% similar to it, why? Because the transition state is highly unstable. So we synthesize compounds that are close to the structure of a transitional state (Transition-State Analog) to inhibit the reaction. (If what's synthesized is so close to the substrate structure we call that a substrate analog.)

Example1: Penicillin:- which is an antibiotic .

Example2: Allopurinol:-Allopurinol a drug used to treat gout النقرص, and a xanthine oxidase inhibitor .It inhibits the enzyme xanthine oxidase which is required to convert hypoxanthine to xanthine in the pathway of adenine(adenine is converted to hypoxanthine then to xanthine) and this enzyme is also required to convert xanthine to uric acid in the pathway of adenine and guanine.



Related Topic -Abzymes-

Abzymes are antibodies and all antibodies are proteins which function in binding to antigens and they are attached to the surface of immune cells.

Abzymes are not inhibitors they are antibodies that has the ability to catalyze chemical reactions meaning they can function as enzymes and catalyze reactions.

How are Abzymes generated? (chill just a long story not much science)
We synthesize a substance similar to the transitional state, that is either a substrate analog or a transition-state analog, then we inject this substance into an animal (a rat or a mouse), the substance is a foreign material (antigen) so the animal will create antibodies specific for those antigens (we end up with antibodies that are able to deal with the original transitional state), we take those antibodies and whenever they come across a transition-state (the original) they will start catalyzing reactions.

- Heavy Metals:

They could inhibit enzymatic function either reversibly or irreversibly, the same metal could have both manners of inhibition.

Metals have many ways to inactivate enzymes they can either coordinate with other metals in the active site of an enzyme, or with the functional groups of the amino acids in the active sites of enzymes.

Examples of metals that inhibit the enzymatic function are: Mercury (Hg), Lead (Pb), Aluminum (Al), Iron (Fe) and many others.

Metals in general work nonspecifically unlike other inhibitors, meaning one metal could bind to active sites of a wide range of enzymes; they are not specific for a certain enzyme, so we can't say for example metal Pb inhibits type X of enzymes only.

Example1: Mercury
Mercury inhibits a wide range of enzymes

Example2: Lead

Lead can inhibit enzymes either reversibly or irreversibly.

The Reversible Inhibition: Lead competes with zinc which is found in the enzyme that catalyses the synthesis of heme, (heme is part of the structure of hemoglobin, myoglobin, cytochromes), so lead occupies zinc's place in the active site, and so the enzyme won't be functioning properly> no heme synthesis> causes anemia.

The Irreversible Inhibition: Lead affects enzymes in the CNS by irreversibly and inhibiting them, this result in a **decreased attention span** (can't pay attention or stay focused for a long time) and decreased cognitive abilities. If Lead is present in high concentrations while neural cells are developing (as in the case of children) it can interfere with the neurons maturity and it could cause mental retardation. (There might be other metals as dangerous as Lead but we give it special attention because it can be present in wall's paint which is something we live with everyday). Lead can be highly toxic to children, when paint chips off the walls, children tend to eat the paint because it tastes sweet so the Lead concentration within their bodies goes high and since neural cells in children are

in the developing stages, Lead affects their CNS and makes them subject to mental retardation.

b) Reversable Inhibition:-

Classified into competitive and non-competitive. What characterizes reversible inhibitors? Rapid association and dissociation from the active site unlike the irreversible inhibitors .

- Competitive Inhibition:

They compete with the substrate on the active site of the enzyme where the binding of that inhibitor prevents the substrate from binding .(2 materials compete for the same site so if we increase the concentration of the substrate we're decreasing the chances of the inhibitor binding and vice versa).

What is the effect of competitive inhibitors on the enzyme parameters (Vmax and Km)?

Vmax doesn't change and Km increases.

Explanation:-

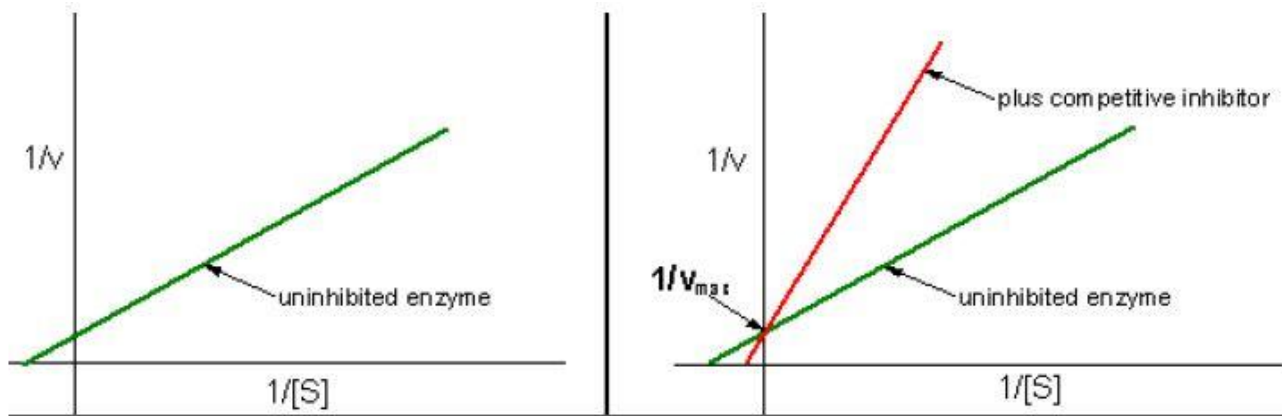
If we add an inhibitor without changing the concentration of the substrate, the enzyme will be catalyzing fewer reactions per unit of time and we'll need more of the substrate concentration to reach 50% of the maximum velocity.

However, if we increase the substrate concentration we increase its chances of binding to the active site and decrease the chances of binding the inhibitor. And upon increasing the substrate concentration more and more we're decreasing the inhibitor chances of binding more and more until we reach a point where we can neglect the effect of the inhibitor. So can we achieve Vmax? Yes we can, because the active site isn't changing its shape or conformation so it can catalyze a full reaction with a full Vmax ,but the km will increase because we need more substrate to achieve 50 % of the Vmax.

<u>**In other words :-**</u> Vmax remains the same <u>because the inhibitor can be overcome by higher substrate concentration</u>

Km which is the substrate concentration need to reach Vmax/2 increases with the presence of a competitive inhibitor <u>because the concentration of the substrate needed to achieve Vmax in the presence of an inhibitor is higher than the Concentration without an inhibitor</u>

we can reach Vmax all the time with competitive inhibitors by increasing the substrate conc.



Intersection points with the Y axis represent the value of (1/Vmax)

The slope represents Km/Vmax

The Lineweaver-Burk plots above shows 2 plots, one for an uninhibited enzyme and the other for the same enzyme but with a competitive inhibitor.

Notice in the plot to the right, the Y intercept is the same for both lines (whether for the inhibited enzyme or the uninhibited enzyme), meaning the value of 1/Vmax doesn't change and so does the value of Vmax. However, the slope changes because km is changing. If we have multiple concentrations of inhibitors we'll see multiple lines, where each line represents a different concentration of the inhibitor, but all lines would have the same Y intercept.

- Non-competitive Inhibition:

This inhibitor binds to a site other than the active site in simple enzymes (composed of one subunit or one polypeptide chain) and after binding it will change the shape of the active site, decreases its affinity towards the substrate, so now it catalyzes the same reactions but with less efficiency and more time.

What is the effect of competitive inhibitors on the enzyme parameters (Vmax and Km)?

Vmax decreases, Km doesn't change

Explanation:

Vmax: the reactions catalyzed per unit of time.

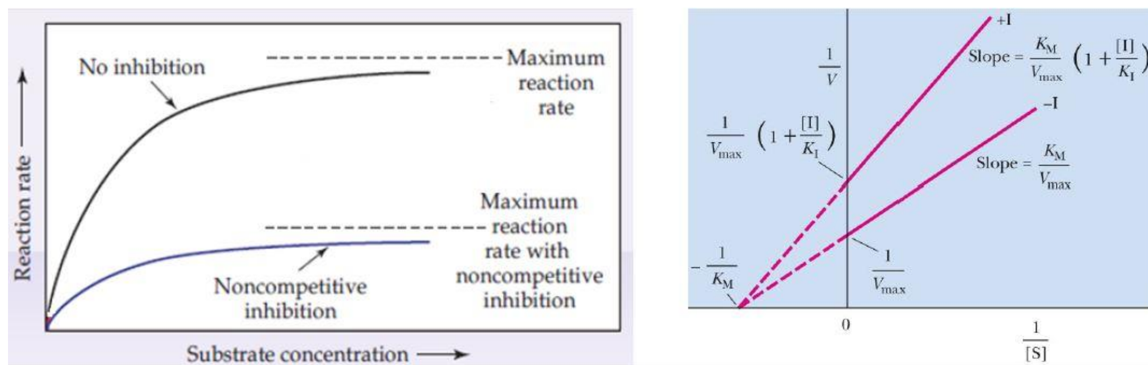
Can we reach Vmax/ within the same unit of time can we achieve the same velocity (can we catalyse the same number of reactions per unit of time)? No, if we increase the substrate concentration will we achieve Vmax? We achieve it if both the substrate and the inhibitor are competing for the same active site, which is not the case here, (here the shape of the active site is changed because the inhibitor binds to a different place other than the active site and thus changes the conformation and the affinity of the active site towards its substrate), so more substrate conc. does nothing. How about if we increase the time? The enzyme will catalyze the same amount of reactions however with more time, (Rate/ velocity= NumberOfRXNs/Unit of time), velocity will decrease for sure (because the denominator مقام الكسر is greater now). So Vmax in non-competitive inhibition will always decrease and be less than that of an uninhibited enzyme.

How about Km of the enzyme? Say we have 10 molecules of the substrate, they need to bind to 10 active sites to have full saturation and to give Vmax, the 50% of the Vmax is achieved for example with 4 molecules of the

substrate. Now with the inhibitor, we change the affinity of the active site towards its substrate so the enzyme will catalyze the 10 reactions (the 10 substrate molecules will bind to the 10 active sites) and the reactions proceed however they will need more time. So in the presence of an inhibitor, how many do we need of the substrate molecules to reach 50% of the vmax? We still need 4 molecules of the substrate to achieve 50% of the NEW Vmax.

The Hall-Guard-Students Example:

Say a lecturer needs 60 students in a hall that has 120 seats for an interactive lecture, 1st scenario: the students were entering the hall easily (wide doors, no one blocking the entrance), say they take 10 mins to enter the hall and be seated, the lecturer achieves the interactivity needed from those 60 students. 2ed scenario: we assign a guard to slow the students down while at the door, or say we make the seats smaller and uncomfortable, how many students does the lecturer need now for an interactive class? Still 60 however the students will take more time to enter the hall and to be seated (say 20 mins).



On a Lineweaver-Burk plot, since Vmax changes, there will be different Y intercepts, one for the uninhibited enzyme and one for the non-competitively inhibited enzyme. However the intersection with the X axis will be the same for all lines because it represents Km.

Remember

Classification of enzymes according to their behavior:

1) Simple Enzymes: Simple enzymes follow michaelis menten equation, they are one-subunit enzymes, they undergo reversible and irreversible inhibition and their inhibitors are either competitive or non-competitive.

2) Allosteric Enzymes: which we'll discuss in a moment.

3) Conformational Modification:

Here the shape of the protein/enzyme is changed.

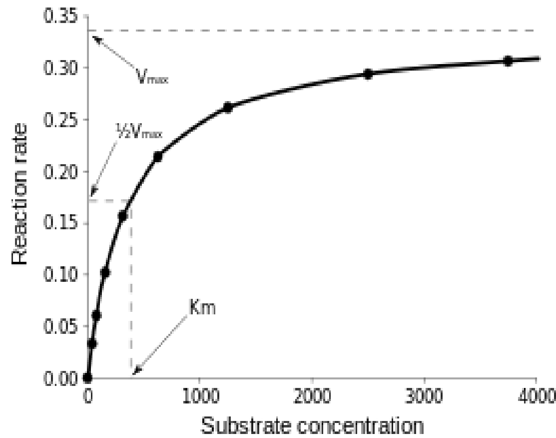
a) Allosteric Enzymes:

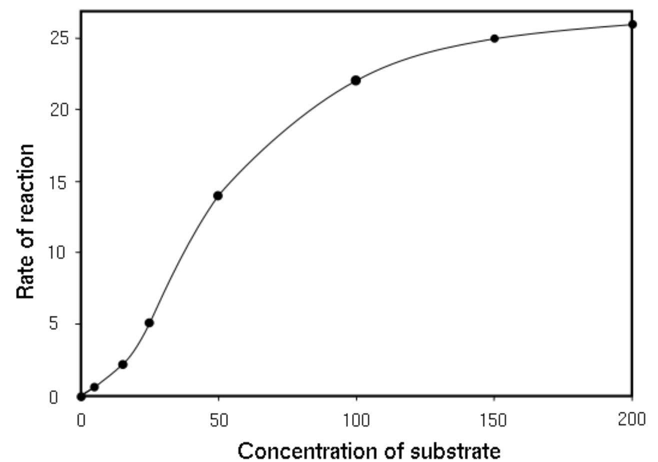
How are they different form simple enzymes?

Allosteric enzymes are multi-subunit enzyme (they have more than 1 polypeptide chain), some of the subunits are regulatory (responsible for enzymatic regulation) others are catalytic (responsible for catalyzing the reactions). A change in one subunit causes a change in enzyme parameters on a different subunit. And unlike simple enzymes, they do not follow the michaelis menten equation and their Lineweaver-Burk plots are not hyperbolic.

Hemoglobin (which is an allosteric protein but not an enzyme) behaves in a manner similar to that of allosteric enzymes, binding oxygen to the first subunit makes it easier for the 2nd subunit to bind oxygen.

Comparing a hyperbolic plot of a simple enzyme with a sigmoidal plot of an allosteric enzyme:





Regarding the 1st plot (the hyperbolic one), with a small increase in the substrate concentration, velocity will highly increase, (1st order reaction kinetics), followed by zero order reaction kinetics where the velocity becomes constant (increases very little) even when we add high concentrations of the substrate.

Regarding the 2nd plot (the sigmoidal one –S shape-), at the beginning, the enzyme has low affinity to the substrate, so high concentrations of the substrate don't catalyze that many reactions, after a while the affinity changes (increases), and small amounts/concentrations of the substrate increase the velocity noticeably, at the end, full saturation of the active sites is achieved and looks like a zero order reaction .

Simple enzymes have a hyperbolic plot, while Allosteric enzymes have a sigmoidal plot.

Fun Fact (not really): Allosteric enzymes have regulatory and catalytic subunits, if we can separate the catalytic from the regulatory, the catalytic subunits of the allosteric enzymes will behave exactly like simple enzymes, because the regulatory subunits that introduce allosterism are gone.

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Homotropic Effectors Vs Heterotropic Effectors:

Homotropic Effectors:

The regulatory molecule is the substrate of the enzyme itself , Going back to hemoglobin as an example: binding of oxygen to the 1st subunit makes it easier for the 2nd subunit to bind oxygen as well. So binding of the substrate to the 1st subunit makes it easier for the substrate to bind with the 2ed subunit, if the binding material that affects the protein/enzyme is the same as the substrate for that protein/enzyme, we call those homotropic effectors because the effecter that makes changes on the other subunits of the enzyme is the same as the substrate.

Heterotropic Effectors:

Here the regulatory molecule is not the enzyme's substrate it is a different molecule For example: if the substrate of an enzyme is CTP and ATP affects the enzyme's parameters when it binds to one subunit of the enzyme, ATP is different from CTP, so we consider ATP working in a heterotropic manner in regulating the enzyme's function because it is not the enzyme's substrate .

Someone asks: There is more than one active site on an allosteric enzyme, right?

Subunits in an allosteric enzyme could have one or more active sites, some subunits don't have active sites at all (the regulatory subunits)

Another Fun Fact:

Simple enzymes have **Km**: which is a parameter that refers to the concentration of the substrate needed to reach 50% of the maximum velocity (Vmax).

Allosteric enzymes have a parameter which refers to the same thing exactly but is called **$K_{0.5}$**

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Allosteric Enzyme+ Inhibitor Vs Allosteric Enzyme+ Activator:

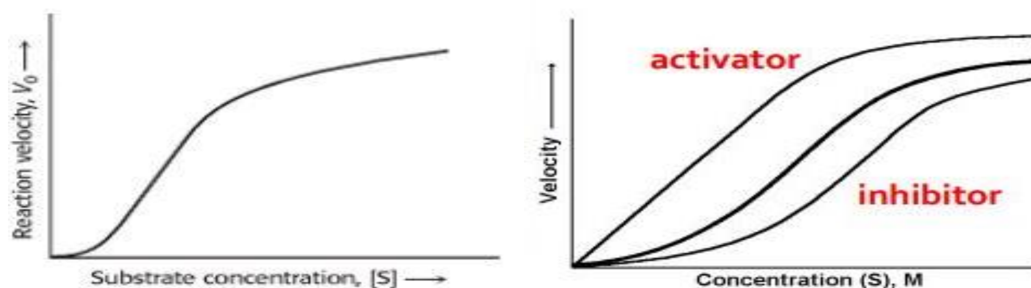
For an uninhibited allosteric enzyme the plot is sigmoidal as mentioned before.

What if we add an allosteric inhibitor?

The plot will shift to the right because we need more concentration of the substrate to achieve the same velocity. So a shift to the right for a sigmoidal curve/plot indicates the addition of an allosteric inhibitor to the enzyme. K0.5 will increase, Vmax will not change and the curve (in the case of an inhibitor) will be more sigmoidal and less hyperbolic. *(plot shift to the right, shape will be more sigmoidal and less hyperbolic, K0.5 increases, Vmax doesn't change).

What if we add an allosteric activator?

The plot will shift to the left and the curve will be more hyperbolic and less sigmoidal.



Any allosteric enzyme can be present in two states/forms: one is called the T state the other is called the R state, meaning there are 2 forms of the enzyme, one with low affinity to the substrate and one with high affinity to the substrate. The one with low affinity is the Tight Conformation of the enzyme, the one with high affinity is the Relaxed Conformation. (Hence the letter abbreviations).

- Are enzymes usually present in their active or inactive conformations?
  Enzymes are usually presented in their inactive forms, we don't want them working as to preserve ATP.
- Which is predominant, the R or the T?
  The T conformation (inactive).
- Would a ratio of (T/R) be high or low?
  Usually, this ratio within physiological conditions would be high.

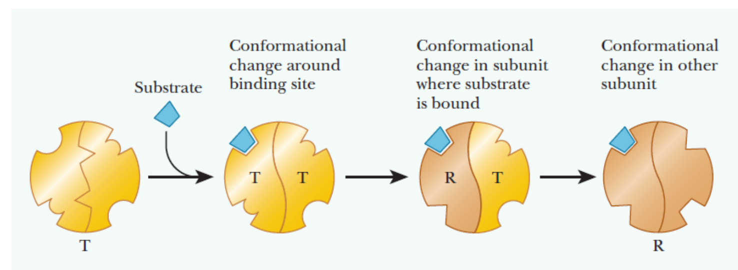
The T/R ratio is called the L ratio, when the value of L increases, it means more of the T (low affinity) and less of the R (high affinity) is present, and so we need more substrate concentration to reach the same velocity (a fixed velocity which before we could reach with less sub conc). Which means the curve will shift to the right. So as L increases the plot will shift to the right and the shape of the curve will become more sigmoidal and less hyperbolic and vice versa .

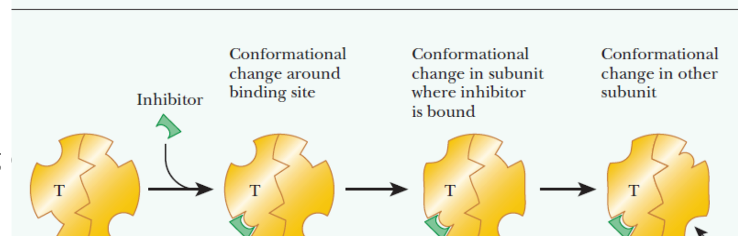
From Active to Inactive

How do enzymes switch from the inactive form to the active form?
For example hemoglobin (a protein but not an enzyme) can be found in the low affinity form for oxygen and the high affinity form for oxygen, how does it change from low affinity (inactive from) to high affinity (active form)?
There are 2 models that explain how Allosteric enzymes work or switch their forms:

1) The Sequential Model التتابعي:

When a substrate binds to the 1st subunit and as a result the 2nd subunit changes its shape/conformation and then the 3rd changes its shape then





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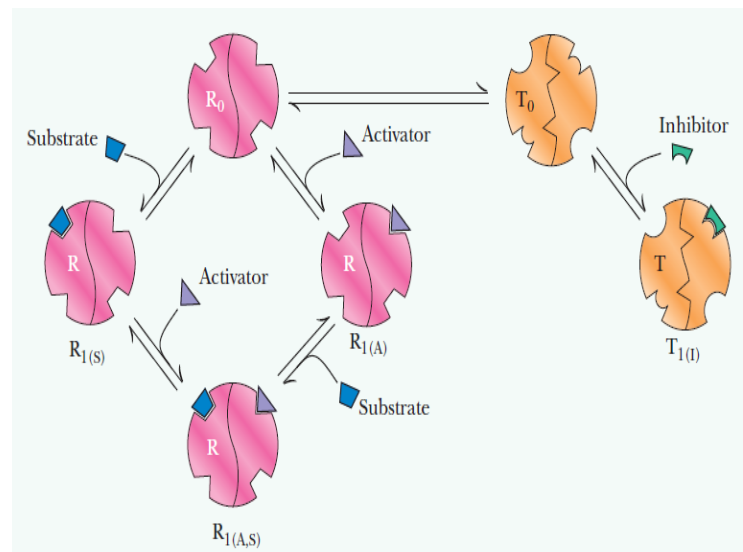
the 4th then the 5th and so on. Like in hemoglobin where the 1st oxygen binds to the 1st subunit, changes the conformation of the 2nd subunit to induce easier binding and better fit for the 2nd oxygen.

*conformational changes are not propagated to all subunits at the same time *

2) The Concerted Model:

States that all subunits of the enzyme change conformation (from low affinity to high affinity and vice versa) simultaneously. So for example, all hemoglobin subunits change from low affinity for oxygen to high affinity for oxygen at the same time. Depending on the substrate concentration, meaning when hemoglobin goes to the lungs where oxygen concentration and pressure are high, that will induce a conformational change in the hemoglobin to convert it to the R state, whereas when hemoglobin reaches the tissues of the body, where oxygen pressure and concentration are low, that will induce a conformational change in the hemoglobin to convert it to the T state.



*Some enzymes and proteins follow the sequential model, some follow the concerted model and some follow both. Hemoglobin for example follows both.
*Note: hemoglobin is not an enzyme, it does not catalyze reactions, it's a binding protein which binds and releases oxygen.

*The T and the R conformation are presented in equilibrium and this equilibrium favors one of these conformations, the equilibrium can be shifted to the T or to the R conformation by the binding of a substrate or allosteric effector to the enzyme , in the concerted model if an activator binds the equilibrium will be

shifted toward the R conformation so we will have more R state than T state which will disrupt this equilibrium .

An example of an allosteric enzyme:

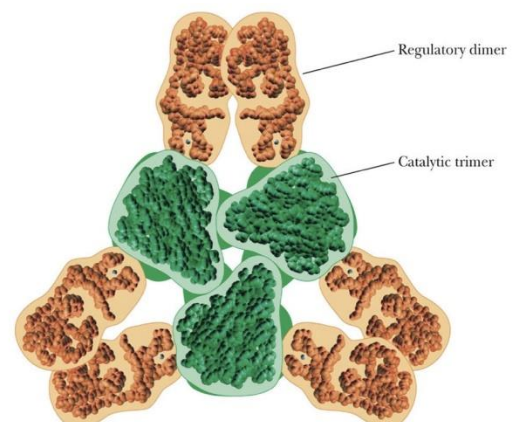
is the ATcase enzyme (Aspartate carbamoyltransferase). The enzyme is responsible for converting Aspartate to CTP and UTP To make DNA and RNA by synthesizing pyrimidines , CTP is the end product of pyrimidine pathway and it works as a feedback inhibitor in allosteric manner for the enzyme. ATP which is the end product of purine synthesis pathway works as an allosteric activator for the enzyme.

Why does CTP work as a feedback allosteric inhibitor? Because when it's concentration is high it means we have high amount of pyrimidines and we need to balance between the amount of purines and pyrimidines so it will inhibits the ATcase enzyme to reduce the amount of pyramidines .

Why does ATP work as an activator? Because ATP is the end product of Purines synthesis so if we have high amount of purines we will also need high amount of pyrimidines to make the DNA and RNA molecules (they are complementary to each other)so high amount of ATP will activate ATcase enzyme to synthesis more pyrimidines . *CTP is the product of the enzyme and will work as a feedback inhibitor.*

The curve of the uninhibited enzyme has a sigmoidal shape, with CTP (inhibitor) it shifts to the right and the shape of the curve will become more sigmoidal and less hyperbolic, with ATP (activator) the curve will shift to the left and will become more hyperbolic and less sigmoidal.

The structure of the ATcase enzyme: 6 regulatory subunits (orange) and 6 catalytic subunits (green). If the regulatory are separated from the catalytic (and this is an experiment that has been done), and the catalytic ones alone are given Aspartate without the presence of the regulatory



subunits, the shape of the plot/curve will be hyperbolic meaning the catalytic subunits will function as simple enzymes.

b) Covalent Modification:

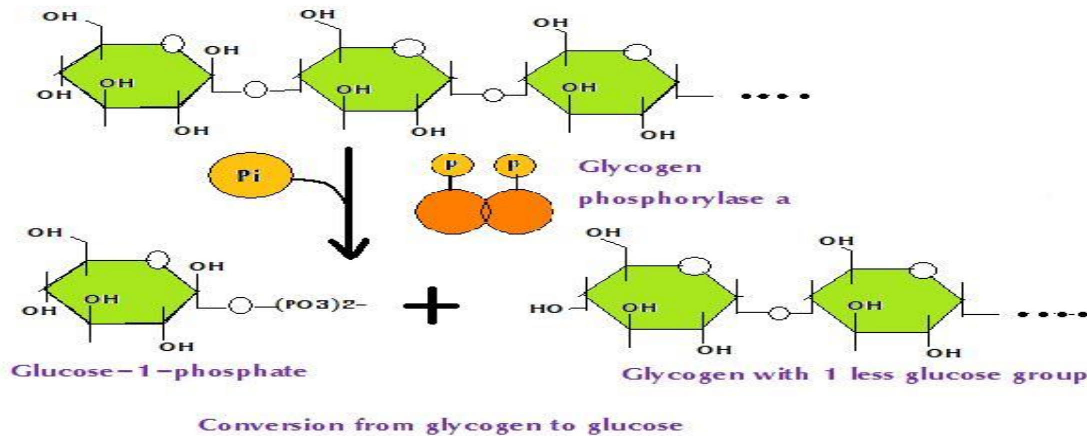
- Phosphorylation is the most common way of covalently binding enzymes.
- Phosphorylation: adding or removing a phosphate group to or from the enzyme.
- The enzymes which add a phosphate group are called kinases.
- The enzymes which remove a phosphate group are called phosphatases.
- ATP is the most common donor of phosphate.

Question:
Does adding a phosphate group to an enzyme activate or inhibit the enzyme?
It depends; it could either inhibit or activate the enzyme, so phosphorylation doesn't necessarily mean enzyme activation.

Question:
When an enzyme (which is a protein with amino acids) is phosphorylated where is the phosphate group added? Where does it bind?
Phosphate groups bind to the oxygens of either a serine, threonine or tyrosine residue in the protein.

Example: (in short)

- The phosphorylation cascade processes that result in freeing glucose form glycogen.
- 2 enzymes -one is called **(glycogen phosphorylase)** the other is **(glycogen phosphorylase kinase)**- are examples of enzymes regulated by phosphorylation.
- Function of **(glycogen phosphorylase)**: it frees glucose from glycogen polymers by adding a phosphate group to the last glucose residue in the glycogen polymer, breaks the bond and frees glucose in the form of glucose-1-phosphate and provides the body with the glucose needed.
- Function of **(glycogen phosphorylase kinase)**: it phosphorylates **glycogen phosphorylase** and makes it active in order to release glucose-1-phoshate from glycogen .



Conversion from glycogen to glucose

<u>The Example (in details):</u> (this is super long but super easy I promise)

*When do we need glucose? When does the body break down glycogen to provide glucose for the body?
When the glucose concentration in the body is low.

*When would the concentration of glucose be low in our bodies?
When we're doing exercise or when we go through stressful situations.

*remember: When we're exercising or stressed, the conc. of ATP in the body will be low, while the conc. of AMP will be high. So AMP works as an activator and ATP works as an inhibitor for glycogen phosphorylase kinase enzyme.

- What enzyme phosphorylates the enzyme (glycogen phosphorylase)?
  The enzyme: glycogen phosphorylase kinase. (this activates glycogen phosphorylase)
- What enzyme dephosphorylates the enzyme (glycogen phosphorylase)?
  The enzyme: glycogen phosphorylase phosphatase also known as Phosphatase1 (this inactivates glycogen phosphorylase)

The enzyme that phosphrylates glycogen phosphorylate which is glycogen phosphorylate kinase, is regulated (made active) by different molecules:
1) AMP (small molecule)

2) Protein Kinase A (large molecule) (we're going to discuss this one)
3) Ca++ calmodulin (large molecule)

The phosphorylation cascade outline:
1) Protein kinase A will be activated
2) Protein kinase A will phosphorylate the enzyme (glycogen phosphorylase kinase).
3) (Glycogen phosphorylase kinase) will phosphorylate the enzyme (glycogen phosphorylase).
4) (Glycogen phosphorylase) is then activated, and then works on glycogen to free glucose.

CyclicAMP and Protein Kinase A

- cyclicAMP/ cAMP: a product of the hormonal cascade and a second messenger within cells, it's derived from ATP
- Protein kinase A: an enzyme which structure consists of 2 regulatory subunits and 2 catalytic subunits, when all these 4 subunits are bound together the enzyme is inactive. It's activated when a material (cAMP) binds to the regulatory subunits of the enzyme.
- Function of cAMP: it activates the enzyme (Protein kinase A)
- 4 molecules (at least) of cAMP bind to the regulatory subunits of protein kinase A, this will separate the regulatory subunits from the catalytic subunits, the catalytic subunits of the protein kinase A are now activated.
- Function of Protein kinase A: phosphorylation, it will phosphorylate (glycogen phosphorylase kinase) so adds a phosphate group to the glycogen phosphorylase kinase and thus activates it.
- Function of (glycogen phosphorylase kinase): phosphorylates glycogen phosphorylase by adding a phosphate group to it and thus activates it.
- Finally glycogen phosphorylase works on glycogen polymers to free glucose.

Summary of what happens:

cAMP→ activates → Protein Kinase A → phosphorylates →
Glycogen Phosphorylase Kinase →Phosphoarylates → Glycogen Phosphorylase
→ adds phosphate to the glucose residues in glycogen → breaks the bonds and frees glucose.

When the concentration of epinephrine/adrenaline (stress hormone) increases, the conc. of cAMP will increase, while the conc. of ATP will decrease. Binding cAMP to the regulatory subunits of protein kinase A causes the release of the catalytic subunits of the protein kinase A (activates the protein/ the subunits), then (Protein kinase A) phosphorylates the enzyme (glycogen phosphorylase kinase), and the long story again.

c) Protein- protein Interactions:
Binding a protein to another causes conformational changes. (we'll discuss this in details next lecture).

d) Proteolytic Cleavage:

Where a part of the enzyme is cut irreversibly in order for the enzyme to be activated, Examples: 1) Chymotrypsinogen: when a part of it is broken it becomes chymotrypsin (the 1st is a precursor). 2) Pepsinogen: when a part is cut/broken it becomes Pepsin. (we'll discuss this in details next lecture).

Good Luck

Special thanks go to Dania Tobasy, Layan Attilli, Noor Yousef and Rafa'a AlMa'ani for helping out in this one.

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