III.8. Allosteric enzymes

• When a protein is made up of several chains of amino acids, each of these chains is a subunit of the protein. The spatial arrangement of the subunits constitutes the quaternary structure of the protein. Allostery concerns proteins with activity: enzymes, transporters, channels, pumps, receptors, contractile proteins, etc.
• Allostery refers to a variation in the conformation of proteins under the effect of the binding of a substrate or an effector molecule, resulting in the acquisition of particular properties (change of activity). This is described as cooperative effects. The binding of the substrate or the effector on one subunit results in an effect on the other subunits of the protein.
• Allosteric effectors are ligands whose binding site is different from the substrate binding site (active site, catalytic site). It may be another substrate or product molecule, different from that which participates in the enzymatic reaction or transport: we then talk about a homotropic allosteric effect. If the effector is a different molecule, we then talk about a heterotropic allosteric effect. The modification of activity is always quantitative: increase, we then talk of allosteric activation or slowing down, or allosteric inhibition.

Example of allostery: myoglobin; hemoglobin

Myoglobin is a protein that transports oxygen into the cytoplasm of cells. It is made up of a single chain of amino acids. Its oxygen transport rate as a function of the pressure of this gas is of the Michaelian type and the curve which represents it is a hyperbola.

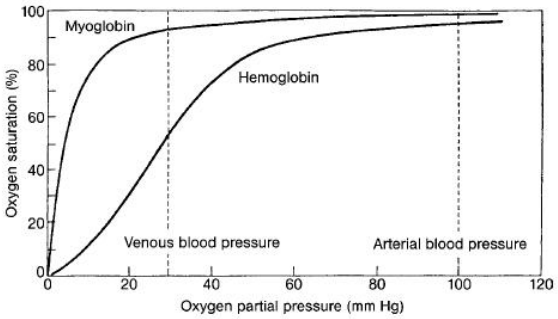


Fig. 1.8 Oxygen saturation curves for myoglobin and hemoglobin (according to M.F. Perutz, *Sci. Am.* 1978, *239*(6), 68–86).

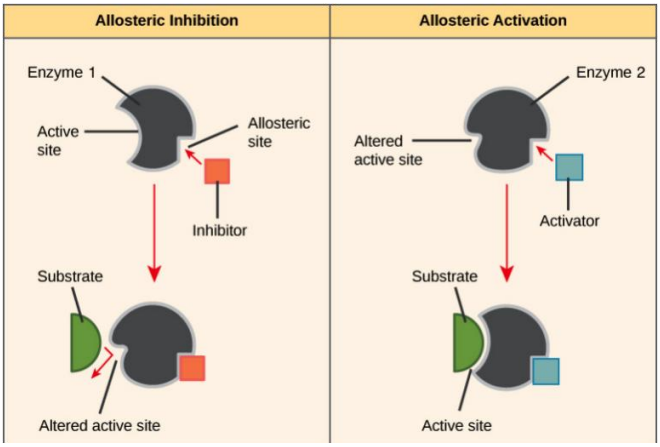
Hemoglobin is a protein that carries oxygen in red blood cells. It is made up of four chains of amino acids. Its oxygen transport rate as a function of the pressure of this gas is of the allosteric type and the curve which represents it is a sigmoid. The cooperation between the protomers gives hemoglobin a high affinity for oxygen in the lungs where it is abundant, and on the contrary a low affinity for oxygen in tissues where it is transmitted to the cells. Hemoglobin therefore behaves differently from one organ to another when the oxygen pressures are different. This protein adapts better to environmental conditions due to allostery.

Allosteric enzymes

Not all enzymes display the velocity–substrate behavior that we have examined so far. One well-known deviation is that displayed by allosteric enzymes. The term allosteric (Greek for other place) indicates that modifiers of the enzyme (effector, also known as a regulator) bind to a place other than the active site at which substrates are converted to products. Their ability to influence the active site remotely, as it were, stems from conformational changes in the protein typically transmitted between separate subunits of a protein.
Many allosteric enzyme participate in cellular metabolism. (eukaryotes – which marks the evolution of these compared to bacteria with regard to metabolic regulation processes). An allosteric enzyme can generally have two different forms: an active form and an inactive form. The effector, by attaching to its allosteric site, has the effect of blocking the enzyme in its active form or in its inactive form (it depends on the enzyme). We then talk of an **inhibitory effector** and an **activating effector**.

- An inhibitory effector has the effect of blocking an enzyme in its inactive form. By binding to the allosteric site, the inhibitory effector modifies the shape of the enzyme which then becomes inactive. As long as the inhibitor is bound to the allosteric site, the enzyme remains in its inactive form. It does not work anymore. If the inhibitor separates from the allosteric site, the enzyme returns to its active form.

- An activating effector makes a normally inactive enzyme active by binding to its allosteric site. When the activator binds to the allosteric site, the enzyme takes its active form. If the activator separates from the enzyme, the latter returns to its inactive form. This type of enzyme only works if it is linked to its effector Without the effector, the enzyme is useless.



Allosteric inhibitors modify the active site of the enzyme so that substrate binding is reduced or prevented. In contrast, allosteric activators modify the active site of the enzyme so that the affinity for the substrate increases.

Most cooperative enzymes share a few features in common. These include:

1. Allosteric enzymes generally consist of multiple subunits (i.e., they are oligomeric).

2. The regulatory ligands (effectors) usually do not share any structural resemblance to the substrate(s) or product(s) of the enzyme reaction concerned.

3. Effectors may bind to an allosteric site distinct from the enzyme active site. It is thus possible to selectively destroy (by physicochemical or mutational methods) the allosteric site without affecting the catalytic site. Such a *desensitized* enzyme does not respond to allosteric effectors. For instance, upon limited heat treatment, *E. coli* aspartate transcarbamoylase loses its ability to bind CTP.

4. Allosteric enzymes do not show Michaelian substrate saturation kinetics. Their v→[*S*] plots are sigmoidal rather than being hyperbolic. The sigmoid saturation curve indicates cooperative substrate binding – thebinding of the first molecule facilitates the binding of subsequent molecules.

The extent of cooperativity is measured by the value of **h** – the Hill coefficient (also denoted as $\mathbf{n_H}$). An enzyme with h = 1 shows no cooperativity and is Michaelian.

If h = n for an enzyme with n binding sites (each monomer with an active site) then such an enzyme will be extremely cooperative.

The model shown in the Fig. 1 is one possible mechanism. Substrate binding alters subunit conformations, indicated in the figure as a transformation from spheres to blocks.

The blocks represent an increased catalytic ability for the subunit. This behavior is called positive cooperativity, an idea borrowed from the equilibrium hemoglobin-oxygen binding curve.

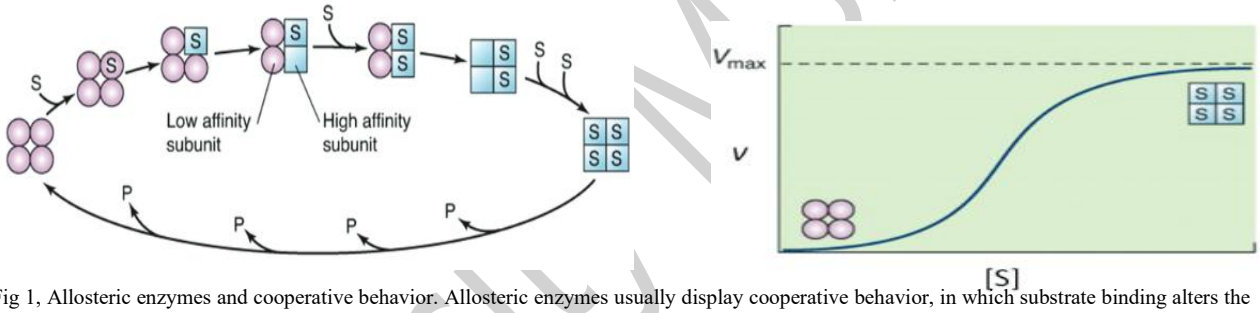


Fig 1, Allosteric enzymes and cooperative behavior. Allosteric enzymes usually display cooperative behavior, in which substrate binding alters the ability of the enzyme to bind further substrate molecules, reflected in a steadily increasing velocity. Eventually, the enzyme is saturated, and velocity falls off to Vmax , as with all enzymes (Ochs, 2022),

Inhibitors of cooperative enzymes shift the curve to the right because they decrease the relative effectiveness of the substrate on v, as indicated in Fig.2. Also shown in the Fig is the shift of the curve to the left, the response to an allosteric activator.

The earliest physical model to account for the behavior of allosteric proteins and enzymes was proposed by Monod, Wyman, and Changeux (Monod et al. 1965).

According to this model, in an oligomeric allosteric enzyme, the subunits occupy equivalent positions within the oligomer. Each monomer can exist in one of the two conformational states: either the R (for relaxed – an active, high-affinity state with tighter binding to the ligand) or the T (for tense – an inactive, low-affinity state with weak/no binding to the ligand) state.

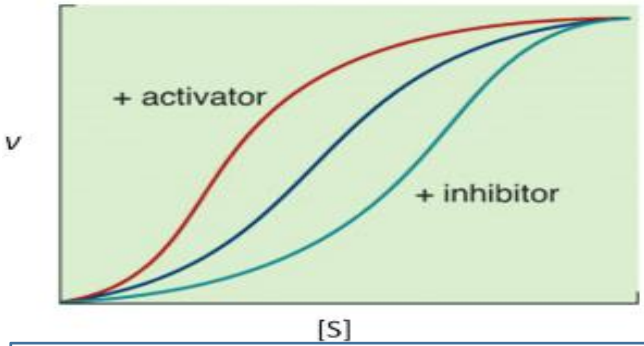
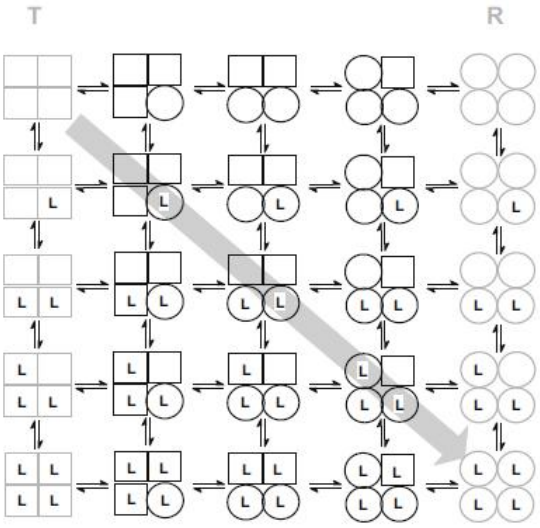


Fig 2, Inhibitors and activators of allosteric enzymes. Modulators of allosteric enzymes cause a shift to the right (inhibitors) or left (activators), greatly extending the influence of these compounds on enzyme velocity over Michaelis–Menten type enzymes (Ochs, 2022).

Further, the monomers are conformationally coupled to each other – when one subunit takes the R conformation, all others also change to R state such that the symmetry of the oligomer is maintained. Hence this model is known as the symmetry model. Allosteric ligands affect the R ⇄ T equilibrium, and the subunits change their conformation in a concerted fashion.

Therefore, it is also called the concerted model. Cooperative binding occurs when the ligand preferentially binds to the R state, thereby displacing the R ⇄ T equilibrium toward the R state. Sigmoidal oxygen binding to hemoglobin is a good example of this model. Nearly 100% of free hemoglobin occurs in T state, while O_2 binds 70 times more tightly to the R state.

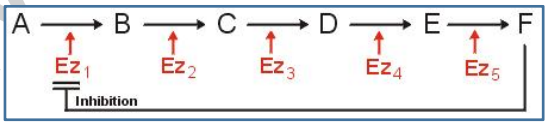


Fig, 3: Models of subunit cooperativity in a tetrameric enzyme. R represents a high affinity form (o) of the tetramer which is in equilibrium with T, the low affinity form (□) of the enzyme.
The two vertical columns (in gray) show the species considered in the Monod, Wyman, and Changeux model. The species occurring along the diagonal (shown by the arrow) represent the forms considered by Koshland, Nemethy, and Filmer model. These two models are special cases of the more general Adair model (that includes all the enzyme species shown) (Punekar (2018)

Koshland, Nemethy, and Filmer proposed another physical model to describe allosteric phenomena – the so-called sequential model (Koshland et al. 1966). This model is based on the concept of ligand binding by "induced fit". In the absence of the ligand, the oligomer exists in one conformational state (and not as equilibrium of R and T states) The subunits change their conformation sequentially as ligand molecules bind (Fig. 3). Conformational change in one subunit alters the interface of that subunit with its neighbors. This may result in more favorable (positive cooperativity) or less favorable (negative cooperativity) binding of the subsequent ligands. Unlike the symmetry model, this model can also account for and explain negative cooperativity.

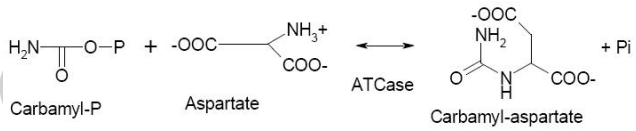
The two models differ in the way ligand binding and conformational states are linked. Accordingly, they make specific predictions as to the allosteric behavior of an enzyme.

Effectors play an important role in controlling the activity of enzymes in the cell. In metabolic chains, the final product obtained at the end of the chain can be an inhibitory effector of an allosteric enzyme at the beginning of the chain.



As the quantity of the final product increases, the reaction that produces it slows down. This is what we call a feedback inhibition control mechanism. If the concentration of F becomes too high in the cell, the metabolic pathway that produces it is then blocked.

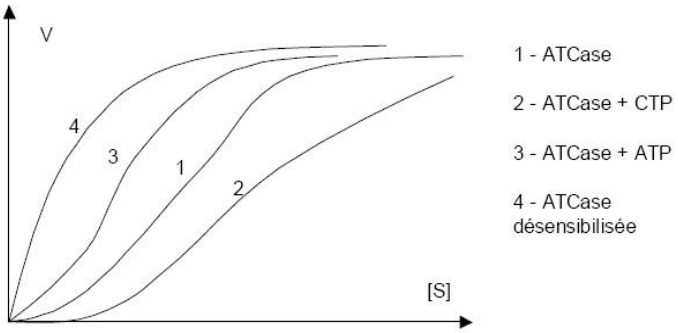
Aspartate transcarbamylase (ATCase) is involved in the regulation of pyrimidine bases.



Kinetic of ATCase

The curves are sigmoid: aspartate plays a role as a positive cooperative effector on the activity of the enzyme: at low substrate concentration, the velocity is not high; the higher the concentration, the more the velocity takes on the appearance of a Michaelis curve

CTP plays a negative role on ATCase: inhibition of activity. In the presence of ATP, there is activation of ATCase: it is a positive effector.



When ATCase is desensitized (by a chemical agent), there is no longer any regulation: the allosteric properties are lost.

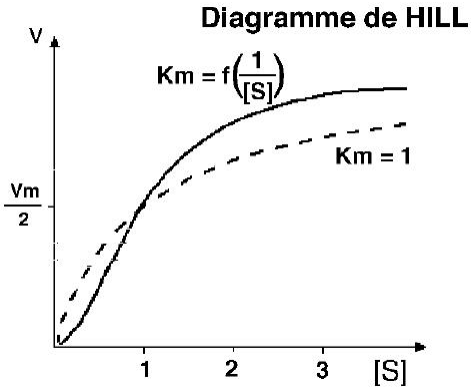
CTP: the basic molecule is cytosine. When too much cytosine is produced, the end-of-chain product will regulate the enzyme upstream of the synthesis pathway: negative feedback.

ATP: when there is a lot of adenine (purine base), there is activation of the synthesis of pyrimidine bases: positive feedback.

Allostery is therefore a tool for fine adjustment, for direct, instantaneous regulation, which is not of the same order as, for example, the activation of the Lactose operon which is a very heavy system.

Diagram of hill

The graph representing the initial velocity of an allosteric enzyme which is not a hyperbola like that of Michaelian enzymes. The rate and affinity constants of such enzymes vary depending on the ligands, such that the curve takes a sigmoid shape, characteristic of the cooperation that takes place between the protomers.

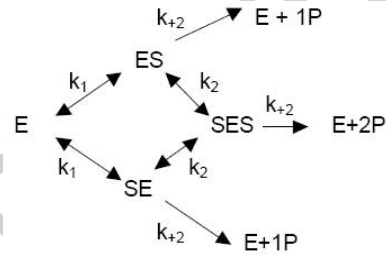


The allosteric kinetics is slower than Michaelian kinetics for small concentrations of the substrate and becomes faster beyond that. Around the inflection point of this sigmoid the slope of the curve is steeper, which means that for the same difference between two concentrations of the substrate, the acceleration of the reaction will be greater in the case of the allosteric enzyme. This property of cooperativity of protomers gives an advantage to allosteric systems compared to enzymes with Michaelian kinetics for regulating the rate of enzymatic reactions.

In the case of allosteric regulation, we have $v = \frac{Vm.S^n}{Kh + S^n}$

with n number of catalytic sites and Kh = k1 x k2 x ... kn

$vKh + vS^n = Vm.S^n \Rightarrow \frac{v}{Vm-v} = \frac{S^n}{Kh} \Rightarrow \log\left(\frac{v}{Vm-v}\right) =$ n log S-log Kh



Case of an enzyme with 2 catalytic subunits

n = 1 : no allosteric regulation

n = 2 : allosteric regulation with 2 catalytic subunits.

n = Hill number = number of allosterically regulated subunits. Examples:

- Phosphofructokinase : n = 4

- Lactate DH : there are 4 subunits, but n = 1: no allosteric regulation.

III.9. Bi-reactant mechanism

Enzymes almost always catalyze reactions with several substrates, frequently two:

$$A+B \longleftrightarrow P+Q$$

Some enzymes require the presence of a dissociable coenzyme. For kinetic analysis, the coenzyme can be formally considered as a second substrate. It happens that the concentration of one of the substrates is in large excess and is not significantly modified during the reaction. In this case, the kinetics can be analyzed taking into account only one substrate. Enzymatic hydrolysis reactions have a second substrate which is water. When it takes place in aqueous solution, the second substrate does not intervene in the kinetics. In another solvent, the concentration of water can be limiting and the kinetic analysis must take this into account.

A Uni Bi mechanism corresponds to a substrate and two products

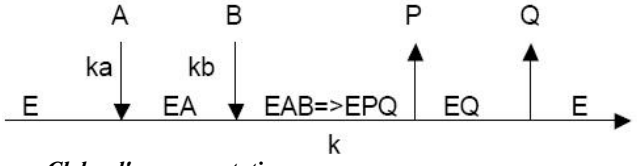
A Bi Bi mechanism, with two substrates and two products

A ter Bi mechanism, with three substrates and two products

A ter Quad mechanism, with three substrates and four products

A. Ordered BiBi mechanism (sequenced association)

• When an enzymatic reaction involves two substrates or a substrate and a free coenzyme, the phases of the enzymatic reaction at the molecular level become complicated: we speak of two-substrate kinetics.



Cleland's representation

• For certain enzymes, a first complex is first formed between the first substrate and the enzyme. This Enzyme-Substrate A complex then forms a complex with the second substrate: Enzyme-Substrate A-Substrate B.

• This ternary complex is then transformed by the action of the enzyme into an Enzyme-Product P-Product Q complex which dissociates by releasing product P and product Q in order.

$$ka = \frac{E.A}{EA} \Rightarrow EA = E.\frac{A}{ka} \qquad kb = \frac{EA.B}{EAB} \Rightarrow EAB = EA.\frac{B}{kb} = E.\frac{A}{ka}.\frac{B}{kb}$$

$$Et = E + EA + EAB = E\left(1 + \frac{A}{ka} + \frac{A.B}{ka.kb}\right) \Rightarrow E = \frac{Et}{1 + \frac{A}{ka} + \frac{A.B}{ka.kb}}$$

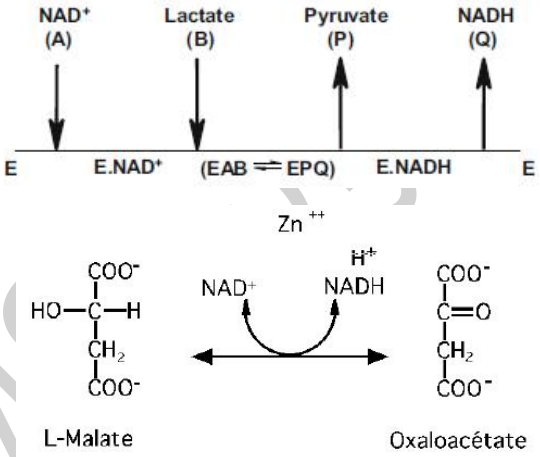
$$\text{or } E = EAB\frac{ka.kb}{A.B} \Rightarrow EAB = \frac{E}{\frac{ka.kb}{A.B}} = \frac{Et}{\frac{ka.kb}{A.B}\left(1 + \frac{A}{ka} + \frac{A.B}{ka.kb}\right)}$$

$$\Rightarrow v = \frac{Vm}{1 + \frac{kb}{B} + \frac{ka.kb}{A.B}}$$

• The free enzyme having no affinity for substrate B, the complex cannot be formed in a different order: this is what justifies the name ordered bibi that we give to this mechanism.

Example of Ordered Mechanism:

Lactate dehydrogenase is an example of ordered bi-substrate reaction. The substrate–product pair of NAD+/NADH is the outer pair and binds the free enzyme according to the scheme.

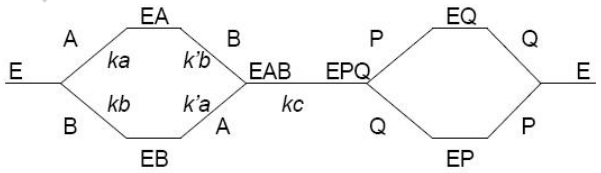


Malate dehydrogenase is an enzyme found in all cells. It catalyzes the oxidation of malate to oxaloacetate by simultaneously reducing a coenzyme NAD+ to NADH. This reaction takes place according to an ordered bibi type mechanism.

the enzyme has no affinity for malate if it is not previously associated with the coenzyme NAD+ in a first complex; then the Enzyme-NAD+-Malate ternary complex is transformed into an Enzyme-NADH-Oxaloacetate complex; the latter complex dissociates, releasing oxaloacetate and then reduced NAD.

B. Random BiBi Mechanism (Random binding)

Other bi-substrate enzymes are capable of forming the Enzyme-Substrate A Substrat B complex by binding the two substrates (or free coenzyme) one after the other but without a fixed order.



the probability of starting with Enzyme-Substrate A or by Enzyme-Substrate B depending only on the respective affinities of the enzyme for these two chemical bodies. This is what justifies the random name given to this mechanism.

Meaning of the constants: ka and kb are the formation constants of the EA and EB complexes, k'a and k'b are the dissociation constants

1) $ka = \frac{[E].[A]}{[EA]} \Rightarrow [EA] = [E]\frac{[A]}{ka}$

2) $kb = \frac{[E].[B]}{[EB]} \Rightarrow [EB] = [E]\frac{[B]}{kb}$

3) $k'a = \frac{[EB].[A]}{[EAB]} \Rightarrow [EAB] = [EB]\frac{[A]}{k'a} = [E]\frac{[B].[A]}{kb.k'a}$

4) $k'b = \frac{[EA].[B]}{[EAB]} \Rightarrow [EAB] = [EA]\frac{[B]}{k'b} = [E]\frac{[B].[A]}{k'b.ka}$

Under initial velocity conditions, [EAB] = constant, hence : 5) k'a.kb = ka.k'b

[Et] = [E] + [EA] + [EB] + [EAB]

$$[Et] = [E]\left(1 + \frac{[A]}{ka} + \frac{[B]}{kb} + \frac{[A].[B]}{k'a.kb}\right)$$

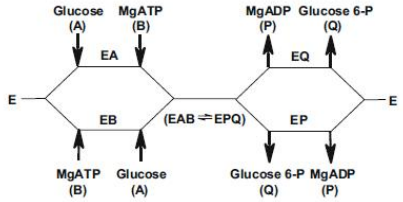
According to relation 4: $[E] = \frac{[EAB]}{[A].[B]}.ka.k'b$,

hence $$[EAB] = \frac{[Et]}{\frac{ka.k'b}{[A].[B]}\left(1+\frac{[A]}{ka}+\frac{[B]}{kb}+\frac{[A].[B]}{k'a.kb}\right)} = \frac{[Et]}{\frac{ka.k'b}{[A].[B]}+\frac{k'b}{[B]}+\frac{k'a}{[A]}+1}$$ (

$$v = \frac{Vm}{1+\frac{k'a}{[A]}+\frac{k'b}{[B]}+\frac{ka.k'b}{[A].[B]}} \text{ ou } v = \frac{Vm}{1+\frac{k'a}{[A]}+\frac{k'b}{[B]}+\frac{k'a.kb}{[A].[B]}}$$

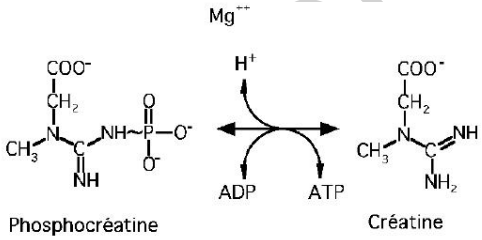
Example of Random Mechanism

Hexokinase is an example of random bi-substrate reaction. The substrates and products bind various enzyme forms according to the scheme.



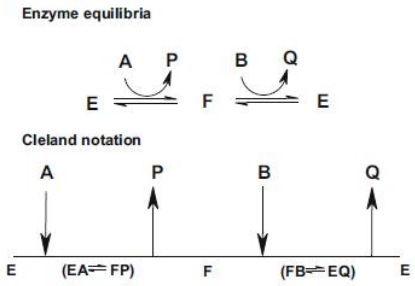
Random bi-substrate reaction of hexokinase

Creatine phosphokinase (CPK) is a vertebrate muscle enzyme. It catalyzes the transfer of a phosphoryl radical from the substrate, creatine phosphate, to a transporter coenzyme, ADP. The affinity of the enzyme for these two chemical bodies being similar, the binding of the enzyme with each of them occurs in an order which depends exclusively on the concentrations.



C. Ping-pong (double displacement) mechanism

The reaction will be catalyzed in two stages. The complex formed between the enzyme and substrate A is first transformed into enzyme + product P, but enzyme E has been chemically modified into enzyme E' during this first part of the reaction. The enzyme E' having an affinity for the second substrate, will form a second Enzyme E'-Substrate B complex which will be transformed into the Enzyme-Product Q complex in a second part of the reaction where the enzyme will regain its initial chemical form . In a two-substrate ping-pong mechanism, no ternary complex (EAB) is formed. A general equation derived for a two-substrate ping-pong case will look like. Ping-pong mechanisms involve double displacements, and a substituted form of the enzyme (denoted as F form) occurs during the catalytic cycle.



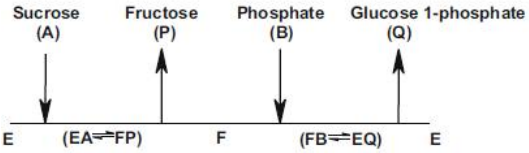
Equilibria representing a bi-reactant ping-pong mechanism

$$v = \frac{V_{max}[A][B]}{K_A[B] + K_B[A] + [A][B]} \qquad v = \frac{Vm}{1+\frac{K_m^B}{B}+\frac{K_m^A}{A}}$$

Example of ping-pong mechanism

Sucrose phosphorylase catalyzes the following reaction.

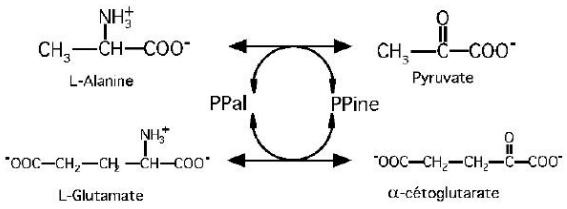
Sucrose + Phosphate ⇄ Fructose + Glucose-1-phosphat



Kinetic scheme for sucrose phosphorylase.

Alanine aminotransferase (ALT) [EC 2.6. 1.2., also called glutamate pyruvate transaminase] catalyzes the transfer of the amine function from alanine to α-ketoglutarate which it transforms into glutamate.

Initially, ALT binds to alanine then transfers the amine function to a linked coenzyme: pyridoxal phosphate which becomes pyridoxamine phosphate without ceasing to be linked to the enzyme. The enzyme then dissociates from the pyruvate. In the second stage, the enzyme linked to the pyridoxamine phosphate forms a complex with α-ketoglutarate, then transfers the amine function of the coenzyme, which becomes pyridoxal phosphate again, to the second substrate which is transformed into glutamate. Finally, the ALT- glutamate complex dissociates: the enzyme and its linked coenzyme have recovered their initial structures.



III. 10. Isoenzymes

Functional structures of enzymes

The tertiary structure (or three-dimensional or conformation) corresponding to the folding of the enzymatic protein is a primordial structure necessary in catalysis. It allows the formation of the active site by bringing together the amino acids constituting it (e.g. AA 57, AA195, AA220). This conformation also plays a role in the protection of the active site. X-ray diffraction has shown that the three-dimensional organization of the active site of enzymes is not entirely the same depending on the absence or presence of the substrate. Thus, the so-called 'induced fit' theory was born where it is stated that enzymes are flexible enough that it can adapt to the shape of the substrate.

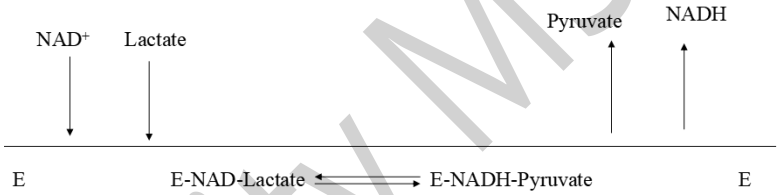
The quaternary structure corresponds to the association of the enzyme into subunits. It gives the final conformational adjustments. This structure leads to the notion of ISOENZYMES (isozymes) which are distinct molecular forms of the same enzyme catalyzing the same reaction, but of which certain physicochemical properties (affinity for S, electric charge, size, regulatory properties) are different. They have in common an action on the same main substrate but different kinetic parameters. Isoenzymes are generally demonstrated by the electrophoresis technique.Isoenzymes are used in the field of labeling animal, plant and microbial genetic resources and in clinical diagnosis.

Lactate dehydrogenase (LDH) (EC 1.1.1.27)is one of the best-known examples. It is a tetrameric enzyme that catalyzes the final step of anaerobic fermentation corresponding to the conversion of lactate to pyruvate.

$CH_3—CO—COOH + 2\ H+ \rightleftharpoons CH_3—CHOH—COOH$

Pyruvate — Lactate

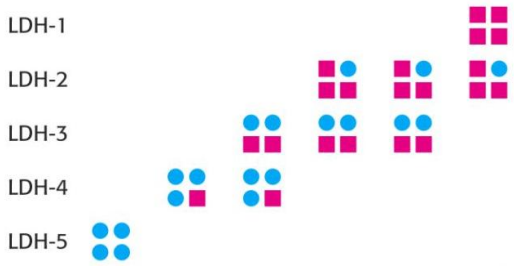
LDH is present in five different forms (isoforms), the proportion of which depends on the nature of the tissue expressing the enzymatic activity. LDH acts in an ordered Bi Bi sequential mechanism or NAD+ first binds to the enzyme.



Beef heart LDH, made up of four homologous subunits, is a typically Michaelian enzyme. LDH is a tetramer that can exist in different forms, resulting from the association of two types of protein chains: the H chain and the M chain.

In muscle, LDH is made up of 4 M-type monomers (for Muscle). The LDH of the muscle is called M4.

In the heart, LDH is made up of 4 H-type monomers (for Heart). The LDH of the heart is called H4.



However, in each of these two organs, we can find the intermediate forms of LDH, in smaller quantities (M3H, M2H2, M1H3). Each of these isoenzymes can be differentiated by their migration position in an electrophoretic field.

Polyenzymatic systems

These are complex protein systems, which can function with several different coenzymes and thus exhibit several activities: they are polyfunctional enzymes. In metabolism the example is pyruvate decarboxylase, a complex comprising 3 enzymes and 5 coenzymes. In such systems, activities are coordinated and catalytic efficiency is increased, by spatial proximity of the substrates, and therefore greater encounter frequencies.

Pro-enzymes (zymogen)

These are enzymes synthesized in inactive form; the activity is only carried out when it loses part of its structure.

Example of some digestive enzymes: