Unit- 2 Enzyme inhibition, coenzymes, Isoenzymes, Uses of enzymes

- **Enzyme inhibition**

From the study of enzyme inhibitors, valuable information can be obtained on the mechanism and pathway of enzyme catalysis, the substrate specificity of the enzymes, and the nature of functional groups at the active site and participation of certain functional groups in maintaining active confirmation of the enzyme molecule. Moreover, inhibition of certain enzymes by specific metabolites is an important element in the regulation of intermediatory metabolism. The enzyme inhibition can be reversible or irreversible.

A) Reversible enzyme inhibition

There are 3 major types of reversible enzyme inhibitions—

I) Competitive
II) Uncompetitive
III) Non-competitive

These 3 types can be experimentally distinguished by the effects of inhibitor on the reaction kinetics of the enzyme, which may be analysed in terms of basic Michaelis-Menten equation. For valid kinetic analysis, the inhibitor must combine rapidly and reversibly with the enzyme.

I) Competitive inhibition

The competitive inhibition is that the inhibitor can combine with the free enzyme in such a way that it competes with the normal substrate for the binding at the active site. A competitive inhibitor reacts reversibly with the enzyme to form an enzyme-inhibitor complex, analogous to the enzyme-substrate complex.

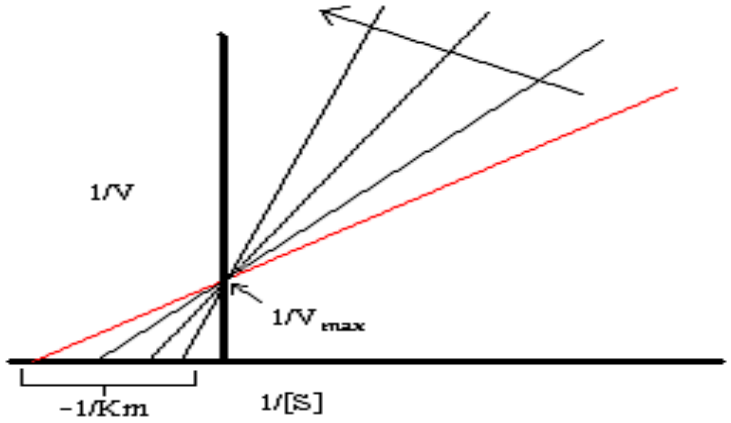
$$E + I \rightleftharpoons EI$$

The inhibitor molecule is not chemically changed by the enzyme. Following the Michaelis-Menten equation, we can define the inhibitor constant KI as the dissociation constant of enzyme-inhibitor complex.

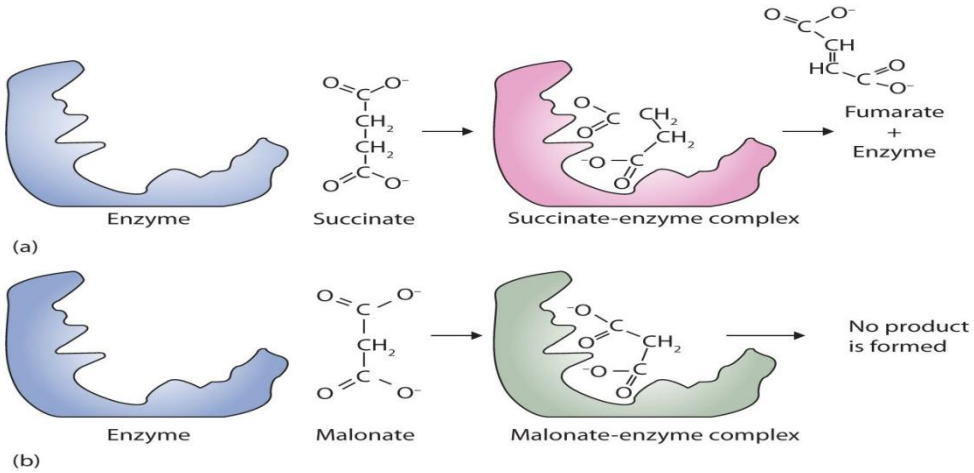
$$KI = \frac{[E]\,[I]}{[EI]}$$

Competitive inhibition is easily recognized experimentally because the percent inhibition at the fixed inhibitor concentration is decreased by increasing the substrate concentration. For quantitative kinetic analysis, the effect of varying substrate concentration [S] on the initial velocity [V_0] is determined at a fixed concentration of inhibitor. This experiment is then repeated with a different concentration of inhibitor; often several series of such experiments are carried out, each at a different concentration of inhibitor. Plots of 1/ V_o versus 1/ [S] are then prepared, one for each concentration of inhibitor. These plots characteristically give a family of straight lines intersecting at a common intercept on 1/ V_0 axis. The presence of competitive inhibitor thus increases the apparent K_M of the enzyme for substrate that is causes it to require a higher substrate concentration to achieve its maximum velocity.

The competitive inhibitor does not affect V_{max} indicating that it does not interfere with the rate of the breakdown of [ES] complex.



The classic example of competitive inhibition is the inhibition of **succinate dehydrogenase** by **malonate and other dicarboxylate anions.**



Some competitive inhibitors of succinate dehydrogenase are malonate, oxaloactate, and pyrophosphates. Note that all contain 2 anionic groups whose spacing resembles that of succinate.

II) Uncompetitive inhibition

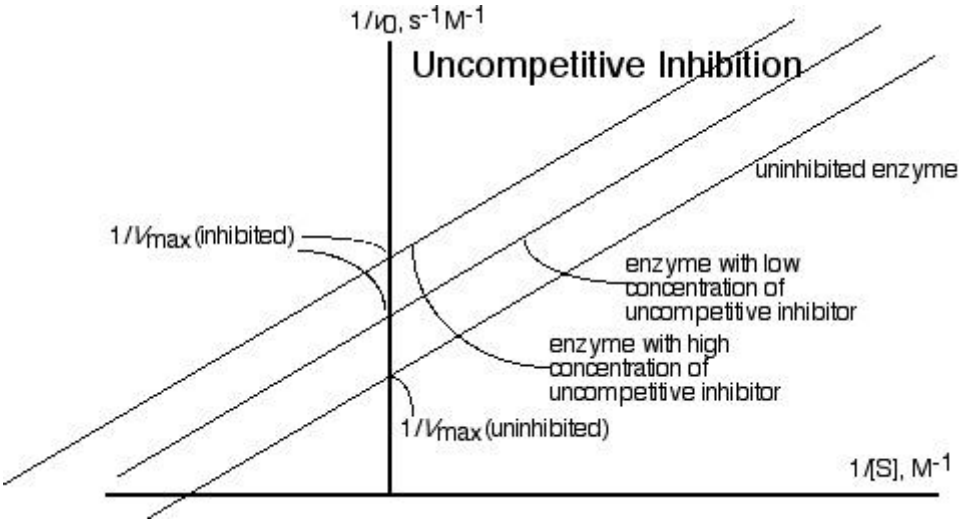
In uncompetitive inhibition, which is not very aptly named, the inhibitor does not combine with the free enzyme or affects its reaction with its normal substrate. However, it combines with enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further reaction to yield the normal product.

$$ES + I \rightleftharpoons ESI$$

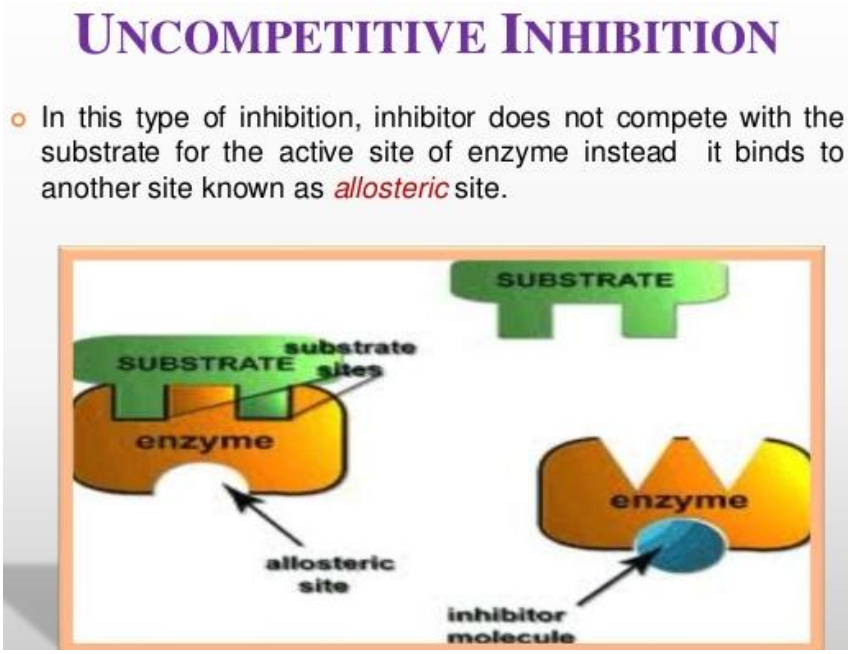
The inhibitor constant is thus

$$KI = \frac{[ES]\,[I]}{[ESI]}$$

These relationships show that the degree of inhibition may increase when the substrate concentration is increased. Uncompetitive inhibition is most easily recognised from plots of $1/V_o$ versus $1/[S]$ at fixed inhibitor concentrations.



As the plot shows, it is typical uncompetitive inhibition that the slope of the plots remains constant at increasing concentrations of inhibitor, but V_{max} decreases. Uncompetitive inhibition is rare in substrate reactions but common in two substrate reactions.



III) Non-competitive inhibition

A non-competitive inhibitor can combine with either free enzyme or enzyme-substrate complex, interfering with action of both. Non-competitive inhibitors bind to a site of the enzyme other than the active site, often to deform the enzyme, so that it does not form the ES complex at its normal rate and once formed, the ES complex does not decompose at the normal rate to yield the products. These effects are not reversed by increasing the substrate concentration. In non-competitive inhibition, the reaction with inhibitor yields two inactive forms EI and ESI.

$$E + I \rightleftharpoons EI$$

$$ES + I \rightleftharpoons ESI$$

The inhibitor constants are

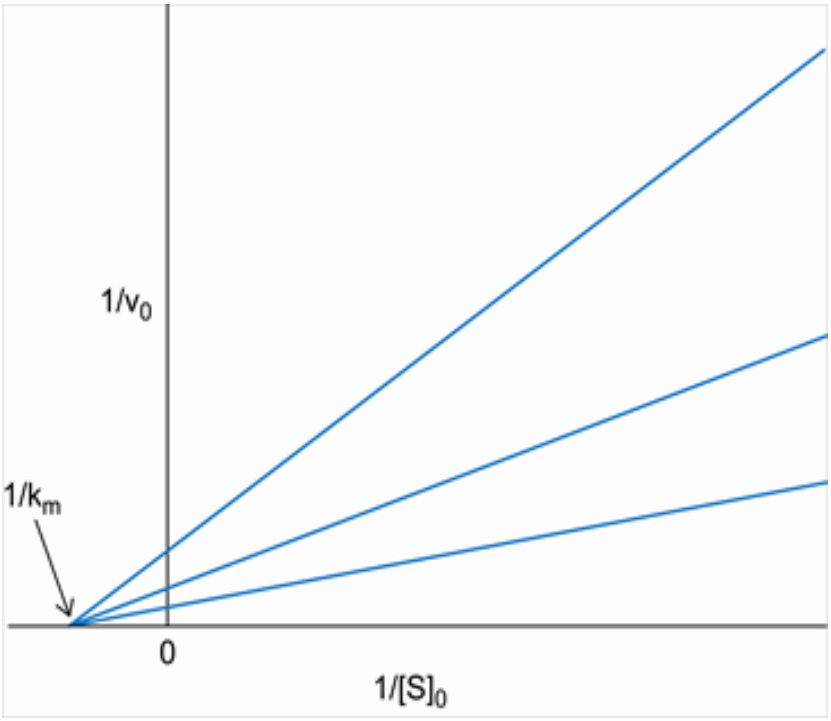
$$K_I^{EI} = \frac{[E][I]}{[EI]}$$

$$K_I^{ESI} = \frac{[ES][I]}{[ESI]}$$

Which may or may not be equal. Non-competitive inhibition is also most easily recognized from 1/ V_o versus 1/ [S] in the presence of different fixed concentrations of inhibitor. The plots differ in the slope but do not share a common intercept on 1/ V_o axis. The intercept on 1/ V_o axis is greater for the inhibited than the uninhibited enzyme, indicating that V_{max} is decreased by the inhibitor and cannot be restored regardless of how high the substrate concentration may be.

The most common type of non-competitive inhibition is given by reagents that can combine with reversibly with some functional groups of the enzymes (outside the active site) that are essential for maintaining the catalytically active 3 dimensional conformation of the enzyme molecule. Some enzymes possessing an essential –SH groups are non-competitively inhibited by heavy metal ions suggesting that such –SH groups must be essential for enzyme to retain its normal active conformation.

Some enzymes that require metal ions for activity are inhibited non-competitively by agents capable of binding the essential metal. E.g. The chelating agents Ethylenediamine tetraacetate (EDTA) reversibly binds Mg^{++} and other divalent cations and thus non-competitively inhibits some enzymes requiring such ions for activity.



B) Irreversible enzyme inhibition (Enzyme modification)

Some enzymes undergo irreversible inactivation when they are treated with agents capable of covalently and permanently modifying a functional group required for catalysis, making the enzyme molecule inactive. This type of inhibition cannot be treated by Michelis-Menten principles, which assumes reversible formation of EI or ESI complexes. Such an irreversible inhibition sets in slowly compared with normal reaction kinetics of the enzyme, so that the inhibition is incomplete at first but continuously increases with time because chemical modification of an increasing fraction of the enzyme molecule takes place.

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❖ **Metabolite antagonism**

A substance that is an antagonist to or resembles a normal human metabolite and interferes with its function in the body, usually by competing for its receptors or enzymes.

Agents produce biological effects by interfering with the action of naturally occurring, physiologically active compounds or with their enzymic transformation, or by blocking the pharmacodynamic action of drugs. Most of these agents achieve their chemotherapeutic effects by interfering in one way or another (often overlapping) with the enzymic processes of the cell.

Among the antimetabolites used as antineoplastic agents are the folic acid analog methotrexate and the pyrimidine analog fluorouracil. The antineoplastic mercaptopurine, an analog of the nucleotide adenine and the purine base hypoxanthine, is a metabolic antagonist of both compounds. Thioguanine, another member of a large series of purine analogs, interferes with nucleic acid synthesis. Cytarabine, used in the treatment of acute myelocytic leukemia, is a synthetic nucleoside that resembles cytidine and kills cells that actively synthesize deoxyribonucleic acid (DNA), apparently by inhibiting the enzyme DNA polymerase.

E is the enzyme, S the substrate, P the product, and KM (Michaelis constant) expresses the substrate concentration at half-maximal velocity (V) and K8 the dissociation constant of ES, the enzyme-substrate complex. The latter can be formed in absence or presence of a coenzyme and/or metal activators dependent on E in question. Prevention of the formation of P is due to inhibitors

acting with E or ES reversibly or irreversibly. In the case of the latter type of antagonism, the agent progressively inhibits the catalytic process which, after a time, is completely arrested.

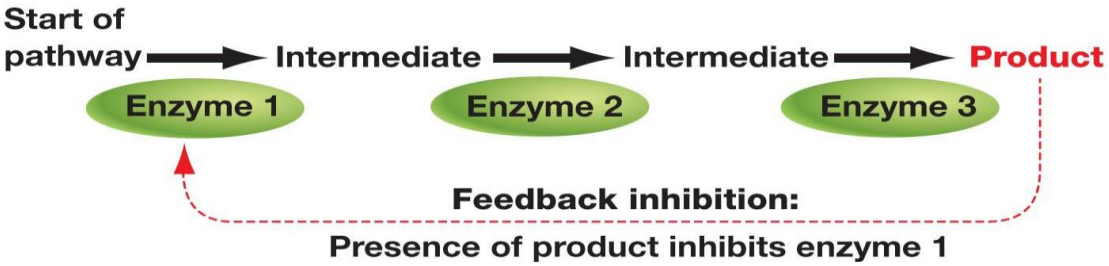
Antagonism can be produced by substrate-like, product or product-like, substrate-and product-unlike and coenzyme- or coenzyme precursor-like compounds. A classical example of a substrate-like inhibitor is that of malonic acid which interferes competitively with oxaloacetic acid decarboxylase and succinic dehydrogenase. An increasing amount of product, either left intracellularly unchanged because of a deficiency of its degrading enzyme or introduced into the biological system from outside, can inhibit the activity of the enzyme necessary for its own synthesis and thus cause a process of negative feedback.

Enzyme antagonists are capable of interacting either with the catalytic or adjacent site or with any parts essential for enzymic activity. Reversible inhibitors in this group are, for instance, aromatic acids, such as salicylic acid, antagonizing and stabilizing bovine xanthine oxidase and m-toluic acid doing the same with d-amino-acid oxidase.

C) FEED - BACK INHIBITION: -

Feedback inhibition occurs when the end product of a reaction interferes with the enzyme that helped produce it. The inhibitor does this by binding to a second active binding site that's different from the one attached to the initial reactant. The enzyme then changes its shape and can't catalyze the reaction anymore. This type of inhibition is done as a regulatory mechanism to meet the metabolic needs of the cell or organism. Many mechanisms, such as bile acid synthesis in the liver and cellular respiration, use feedback inhibition on a regular basis.

Feedback inhibition works by deactivating an enzyme using the product of the reaction the enzyme catalyzes. Enzymes bind to molecules with active sites that are specifically designed to fit with the molecule undergoing the reaction. These enzymes have a second active site for the reaction product to bind to. This causes the enzyme to spatially re-arrange so it can no longer bind to the initial reagent and the reaction stops. Sometimes, the enzymes -- such as pyruvate kinase, which helps break down glucose -- are also chemically modified to halt the reaction.



One purpose of feedback inhibition is to prevent too much of the product from being made. Feedback inhibition balances production of amino acids, the building blocks of proteins. For example, the enzyme threonine deaminase is inhibited by one of its products: the amino acid isoleucine. If the reaction weren't shut off, the enzyme couldn't synthesize other amino acids that the cell needs. However, the reaction restarts when there is not enough isoleucine. To accomplish this, the enzyme binds to another amino acid, valine, which turns the enzyme back on.

Feedback inhibition is also necessary to prevent enzymes from breaking down too many molecules that are energy sources for the cell, such as glucose. Inhibition takes place in glycolysis, the process of breaking down the sugar glucose to produce the cell's "energy currency" molecule ATP. ATP slows down the enzymes until they're structurally modified and stop catalyzing reactions. The enzymes are inhibited when blood glucose levels are low, so there isn't a total depletion and the cell then has a chance to accumulate more glucose for later use.

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❖ **Coenzymes and respective enzymes**

Coenzymes are small organic molecules that link to enzymes and whose presence is essential to the activity of those enzymes. Coenzymes belong to the larger group called cofactors, which also includes metal ions; cofactor is the more general term for small molecules required for the activity of their associated enzymes. The relationship between these two terms is as follows

Cofactors

- Essential ions
- Loosely bound (forming metal-activated enzymes)
- Tightly bound (forming metalloenzymes)
- Coenzymes
- Tightly bound prosthetic groups
- 2 Loosely bound cosubstrates

Many coenzymes are derived from vitamins. Table lists vitamins, the coenzymes derived from them, the type of reactions in which they participate, and the class of coenzyme.

Prosthetic groups

These are tightly bound to enzymes and participate in the catalytic cycles of enzymes. Like any catalyst, an enzyme–prosthetic group complex undergoes changes during the reaction, but before it can catalyze another reaction, it must return to its original state.

Flavin adenine dinucleotide (FAD) is a prosthetic group that participates in several intracellular oxidation -reduction reactions. During the catalytic cycle of the enzyme succinate dehydrogenase, FAD accepts two electrons from succinate, yielding fumarate as a product. Because FAD is tightly bound to the enzyme, the reaction is sometimes shown this way

$$\text{Succinate} + \text{E–FAD} \rightarrow \text{Fumarate} + \text{E–FADH}_2$$

where E–FAD stands for the enzyme tightly bound to the FAD prosthetic group. In this reaction the coenzyme FAD is reduced to $FADH_2$ and remains tightly bound to the enzyme throughout. Before the enzyme can catalyze the oxidation of another succinate molecule, the two electrons now belonging to $E–FADH_2$ must be transferred to another electron acceptor, ubiquinone. The regenerated E–FAD complex can then oxidize another succinate molecule.

Cosubstrates

These are loosely bound coenzymes that are required in stoichiometric amounts by enzymes. The molecule nicotinamide adenine dinucleotide (NAD) acts as a cosubstrate in the oxidation-reduction reaction that is catalyzed by malate dehydrogenase, one of the enzymes of the citric acid cycle.

$$\text{Malate} + NAD^+ \rightarrow \text{Oxaloacetate} + NADH + H^+$$

In this reaction, malate and NAD^+ diffuse into the active site of malate dehydrogenase. Here NAD^+ accepts two electrons from malate; oxaloacetate and NADH then diffuse out of the active site. The reduced NADH must then be

returned to its NAD^+ form. For each catalytic cycle, a "new" NAD^+ molecule is needed if the reaction is to occur; thus, stoichiometric quantities of the cosubstrate are needed. The reduced form of this coenzyme (NADH) is converted back to the oxidized form (NAD^+) via a number of simultaneously occurring processes in the cell, and the regenerated NAD^+ can then participate in another round of catalysis.

Coenzymes

These are a type of cofactor. They are small organic molecules that bind tightly (prosthetic groups) or loosely (cosubstrates) to enzymes as they participate in catalysis.

Table. Vitamins, the coenzymes derived from them, the type of reactions in which they participate, and the type of coenzyme.

VITAMINS AND COENZYMES			
Vitamin	**Coenzyme**	**Reaction type**	**Coenzyme class**
B_1 (Thiamine)	TPP	Oxidative decarboxylation	Prosthetic group
B_2 (Riboflavin)	FAD	Oxidation/Reduction	Prosthetic group
B_3 (Pantothenate)	CoA - Coenzyme A	Acyl group transfer	Cosubstrate
B_6 (Pyridoxine)	PLP	Transfer of groups to and from amino acids	Prosthetic group
B_{12} (Cobalamin)	5-deoxyadenosyl cobalamin	Intramolecular rearrangements	Prosthetic group
Niacin	NAD^+	Oxidation/Reduction	Cosubstrate
Folic acid	Tetrahydrofolate	One carbon group transfer	Prosthetic group
Biotin	Biotin	Carboxylation	Prosthetic group

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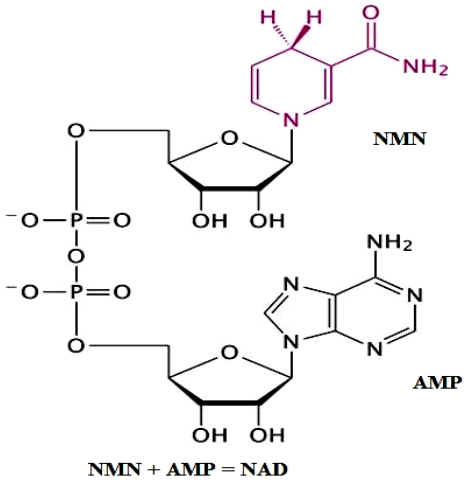
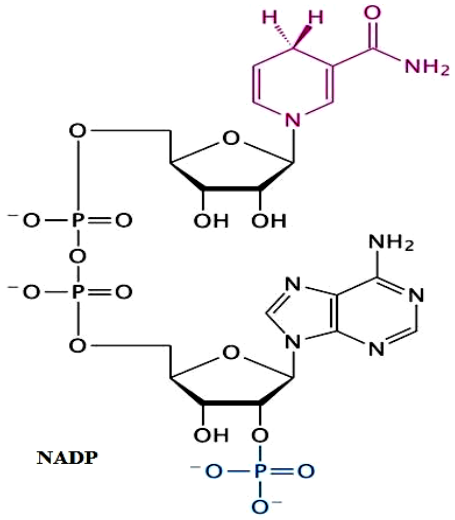
- **NAD (Nicotinamide adenine dinucleotide)**

Nicotinamide adenine dinucleotide (**NAD**) is a coenzyme found in all living cells and is the coenzyme form of the **vitamin** niacin or nicotinic acid (a vitamin of the B complex). The compound is a dinucleotide, because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as **NAD^+** and **NADH** respectively.

Nicotinamide adenine dinucleotide, like all *dinucleotide*s, consists of two nucleotides joined by a pair of bridging phosphate groups. The nucleotides consist of ribose rings, one with adenine attached to the first carbon atom (the 1' position) and the other with nicotinamide at this position.



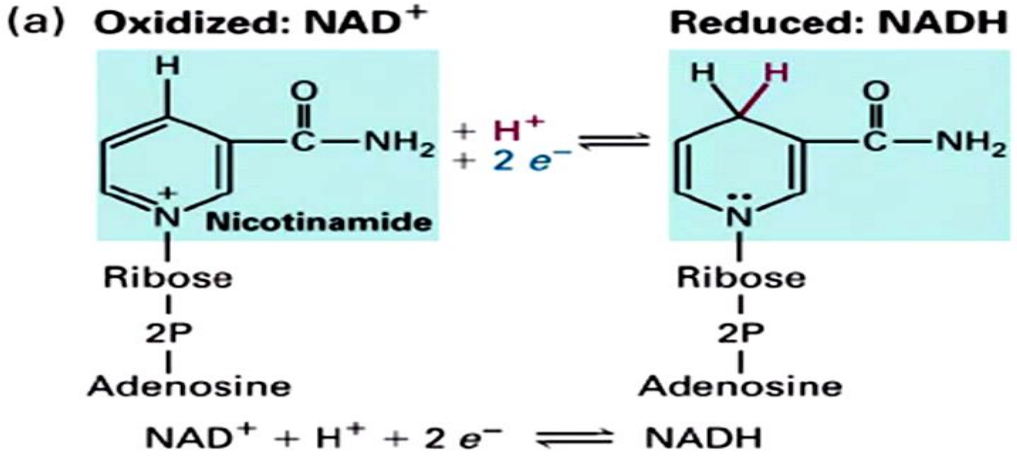


Niacin is a component of two coenzymes: NAD, and nicotinamide adenine dinucleotide phosphate (NADP). NAD^+ (the oxidized form of the NAD coenzyme) is important in **catabolism** and in the production of metabolic energy. $NADP^+$ (the oxidized form of NADP) is important in the **biosynthesis** of fats and sugars.

In metabolism, the compound accepts or donates electrons in redox reactions. Such reactions (summarized in formula below) involve the removal of two hydrogen atoms from the reactant (R), in the form of a hydride ion (H^-), and a proton (H^+). The proton is released into solution, while the reductant RH_2 is oxidized and NAD^+ reduced to NADH by transfer of the hydride to the nicotinamide ring.

$$RH_2 + NAD^+ \rightarrow NADH + H^+ + R$$

From the hydride electron pair, one electron is transferred to the positively charged nitrogen of the nicotinamide ring of NAD^+, and the second hydrogen atom transferred to the C4 carbon atom opposite this nitrogen.



Although NAD^+ is written with a superscript plus sign because of the formal charge on a particular nitrogen atom, at physiological pH for the most part it is actually a singly-charged anion (charge of minus 1), while NADH is a doubly-charged anion.

The adenine, ribose, and phosphate compounds are linked exactly as in the nucleotide molecule adenosine diphosphate (ADP). In the case of NAD $^+$, the nicotinamide ring has a positive charge on its nitrogen atom: This is the + indicated in the designation NAD $^+$. This is often confusing, because the molecule as a whole is negatively charged due to the presence of the phosphate groups, as shown in the figure. A variant of NAD $^+$ form, called NADP $^+$, contains a third phosphate group attached to one of the ribose rings.

In metabolism, nicotinamide adenine dinucleotide is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is, therefore, found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced. This reaction forms NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins, in posttranslational modifications. Because of the importance of these functions, the enzymes involved in NAD metabolism are targets for drug discovery.

ENZYMES THAT REQUIRE NAD AND NADP		
	Enzyme	Function
Enzymes that use NAD^+/NADH	Alcohol dehydrogenase	Metabolizes alcohol
	Glyceraldehyde phosphate dehydrogenase	Catalyzes important step in glycolysis
	Pyruvate dehydrogenase	Catalyzes reactions connecting glycolysis to the Krebs cycle
	NADH dehydrogenase	Catalyzes oxidative phosphorylation reactions
Enzymes that use $NADP^+$/NADPH	Glucose 6-phosphate dehydrogenase	Catalyzes reactions in the pentose phosphate pathway
	β -ketoacyl-ACP reductase β -enoyl-ACP reductase	Catalyzes reactions in fatty acid synthesis
	Chloroplast glyceraldehyde Phosphate dehydrogenase	Catalyzes reactions in the Calvin cycle, glucose synthesis

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- **FAD (Flavin Adenine Dinucleotide)**

In biochemistry, **flavin adenine dinucleotide** (**FAD**) is a redox cofactor, more specifically a prosthetic group, involved in several important reactions in metabolism. FAD can exist in three (or four: see below, flavin-N(5)-oxide) different redox states, which are the quinone, semiquinone, and hydroquinone. FAD is converted between these states by accepting or donating electrons.

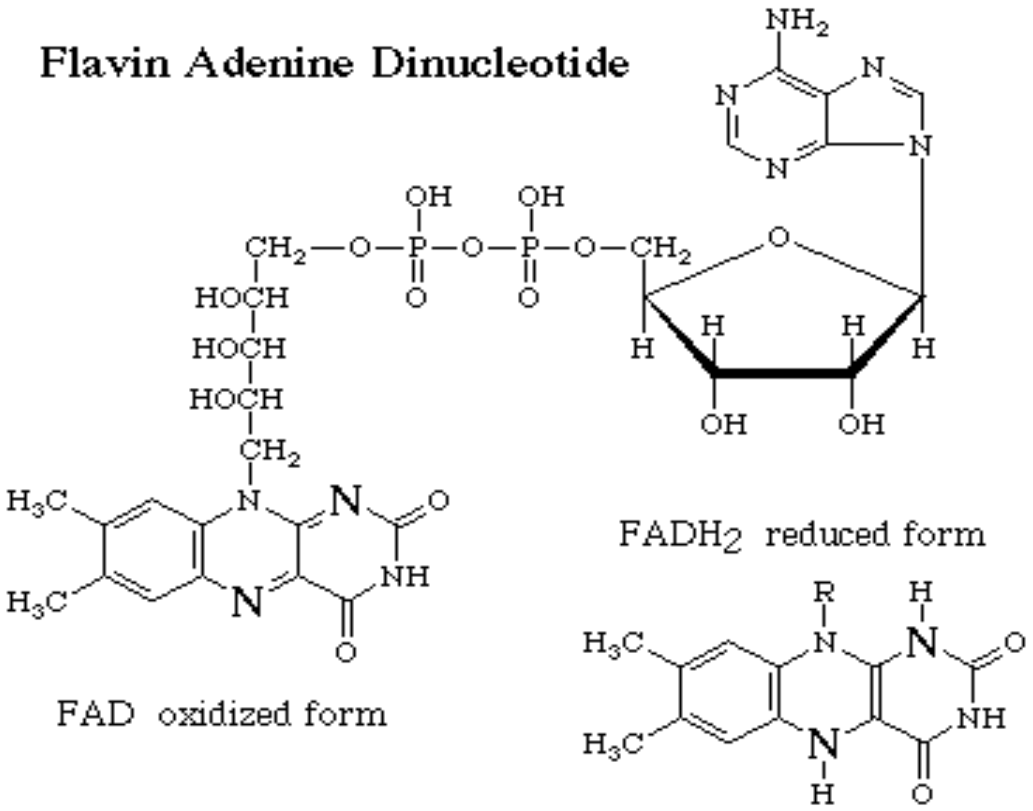
Flavin adenine dinucleotide consists of two main portions: an adenine nucleotide (adenosine monophosphate) and a flavin mononucleotide bridged together through their phosphate groups. Adenine is bound to a cyclic ribose at the 1' carbon, while phosphate is bound to the ribose at the 5' carbon to form the adenine nucledotide. Riboflavin is formed by a carbon-nitrogen (C-N) bond between a isoalloxazine and a ribitol. The phosphate group is then bound to the on the terminal ribose carbon to form a FMN. Because the bond between the isoalloxazine and the ribitol is not considered to be a glycosidic bond, the flavin mononucleotide is not truly a nucleotide.[4] This makes the dinucleotide name

misleading; however, the flavin mononucleotide group is still very close to a nucleotide in its structure and chemical properties.

FAD can be reduced to $FADH_2$ through by the addition of two H^+ and two e^-. $FADH_2$ can also be oxidized by the loss of one H^+ and one e^- to form FADH. The FAD form can be recreated from another loss on one H^+ and one e^-. FAD formation can also occur through the reduction and dehydration of flavin-N(5)-oxide.

FAD (fully oxidized form, or quinone form) accepts two electrons and two protons to become $FADH_2$ (hydroquinone form). The semiquinone ($FADH^{\cdot}$) can be formed by either reduction of FAD or oxidation of $FADH_2$ by accepting or donating one electron and one proton, respectively. See the mechanism section below for details.

A flavoprotein is a protein that contains a flavin moiety; this may be in the form of FAD or flavin mononucleotide (FMN). There are many flavoproteins besides components of the succinate dehydrogenase complex, including α-ketoglutarate dehydrogenase and a component of the pyruvate dehydrogenase complex.



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❖ Lipoic acid

Lipoic acid (LA), also known as α-lipoic acid and alpha lipoic acid (ALA) and thioctic acid is an organosulfur compound derived from octanoic acid. ALA is made in animals normally, and is essential for aerobic metabolism. It is also manufactured and is available as a dietary supplement in some countries where it is marketed as an antioxidant, and is available as a pharmaceutical drug in other countries.

Lipoic acid (LA), also known as α-lipoic acid and alpha lipoic acid (ALA) and thioctic acid is an organosulfur compound derived from octanoic acid. LA contains two sulfur atoms (at C6 and C8) connected by a disulfide bond and is thus considered to be oxidized although either sulfur atom can exist in higher oxidation states.

The carbon atom at C6 is chiral and the molecule exists as two enantiomers (*R*)-(+)-lipoic acid (RLA) and (*S*)-(-)-lipoic acid (SLA) and as a racemic mixture (*R*/*S*)-lipoic acid (R/S-LA).

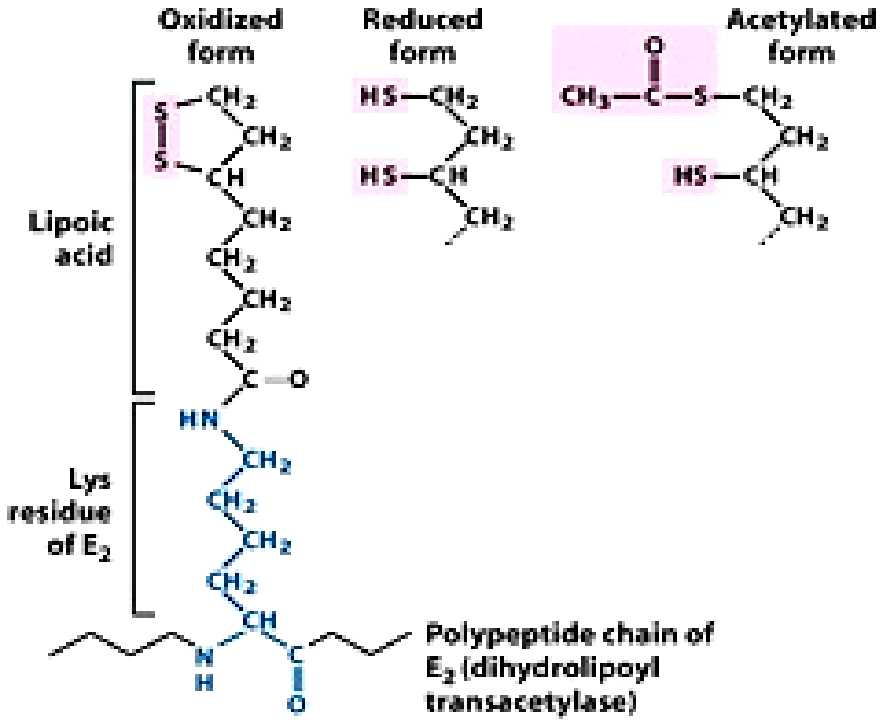
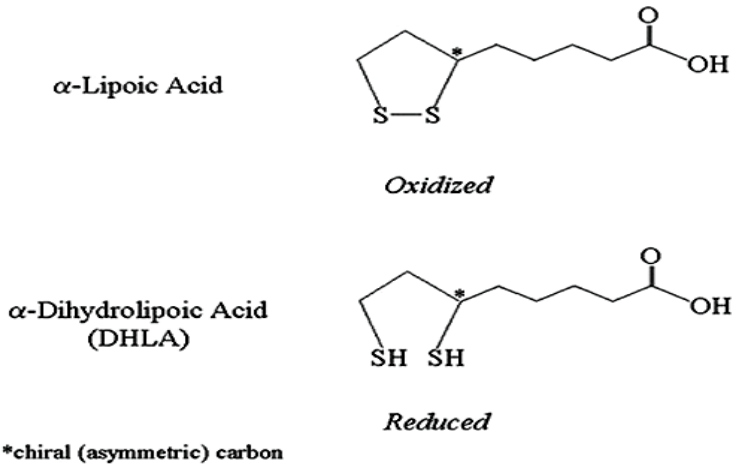


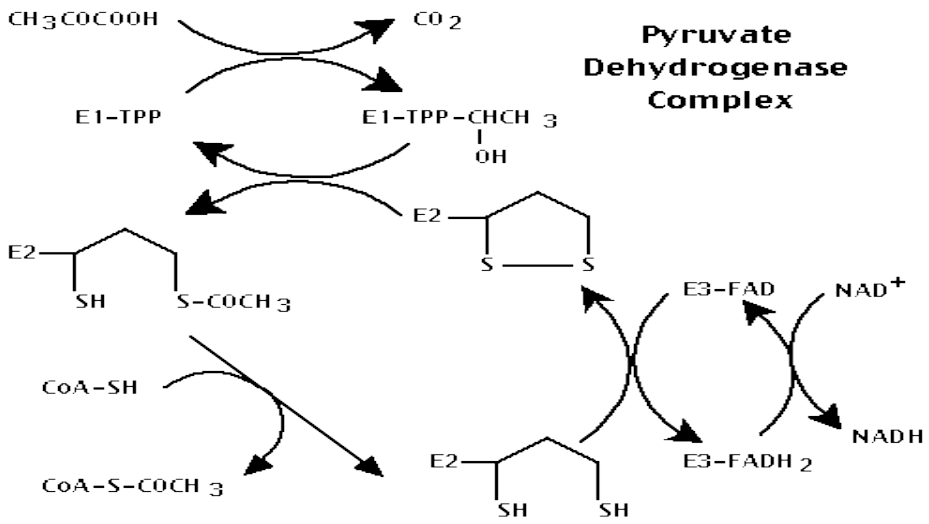
Figure 1. Chemical Structure of Lipoic Acid



*Lipoic has a chiral center, which means it can be found in two mirror image forms (S- and R-α-lipoic acid) that cannot be superimposed on each other.

Lipoic acid is cofactor for at least five enzyme systems. Two of these are in the citric acid cycle through which many organisms turn nutrients into energy. Lipoylated enzymes have lipoic acid attached to them covalently. The lipoyl group transfers acyl groups in 2-oxoacid dehydrogenase complexes, and methylamine group in the glycine cleavage complex or glycine dehydrogenase.
2-Oxoacid dehydrogenase transfer reactions occur by a similar mechanism in:

1. The pyruvate dehydrogenase complex.
2. The α-ketoglutarate dehydrogenase or 2-oxoglutarate dehydrogenase complex.
3. The branched-chain oxoacid dehydrogenase (BCDH) complex.
4. The acetoin dehydrogenase complex.



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❖ Vitamin B_{12} (Cyanocobalimin)

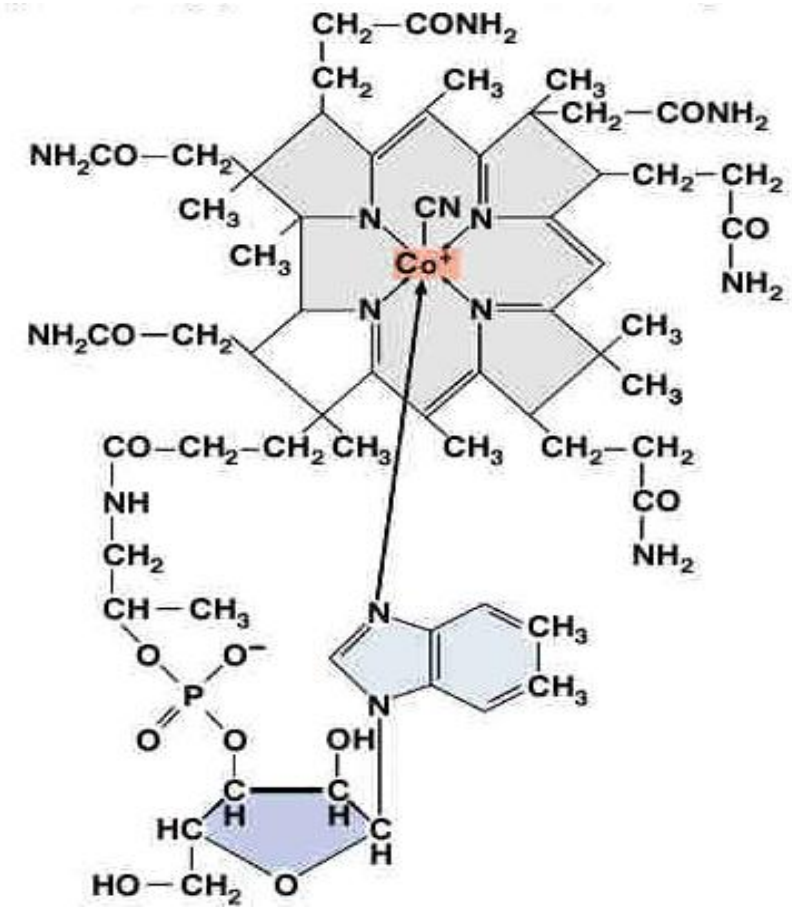
Vitamin B_{12} is also called cynocobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism. Neither fungi, plants, nor animals (including humans) are capable of producing vitamin B_{12}. Only bacteria and archaea have the enzymes required for its synthesis, although many foods are a natural source of B_{12} because of bacterial symbiosis. The vitamin is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.

Vitamin B_{12} deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be experienced.

Vitamin B_{12} deficiency can also cause symptoms of mania and psychosis. Vitamin B_{12} was discovered from its relationship to disease pernicious anemia, which is an autoimmune disease in which parietal cells of the stomach responsible for secreting intrinsic factor are destroyed (these cells are also responsible for secreting acid in the stomach). Because intrinsic factor is crucial for the normal absorption of B_{12}, its lack in pernicious anemia causes a vitamin B_{12} deficiency.

Vitamin B_{12} is the most chemically complex of all the vitamins. The structure of B_{12} is based on a corrin ring, which is similar to the porphyrin ring found in heme, chlorophyll, and cytochrome. The central metal ion is cobalt. Four of the six coordination sites are provided by the corrin ring, and a fifth by a dimethylbenzimidazole group. The sixth coordination site, the center of reactivity, is variable, being a cyano group (-CN), a hydroxyl group (-OH), a methyl group (-CH_3) or a 5'-deoxyadenosyl group (here the C5' atom of the deoxyribose forms the covalent bond with Co), respectively, to yield the four B_{12} forms mentioned below. Historically, the covalent C-Co bond is one of first examples of carbon-metal bonds to be discovered in biology. The hydrogenases and, by necessity, enzymes associated with cobalt utilization, involve metal-carbon bonds.

Vitamin B_{12} is a generic descriptor name referring to a collection of cobalt and corrin ring molecules which are defined by their particular vitamin function in the body. All of the substrate cobalt-corrin molecules from which B_{12} is made must be synthesized by bacteria. However, after this synthesis is complete, except in rare cases, the human body has the ability to convert any form of B_{12} to an active form, by means of enzymatically removing certain prosthetic chemical groups from the cobalt atom, and replacing them with others.



Reactive C-Co bond of Coenzyme B_{12} participates in three main types of enzyme-catalyzed reactions.

1. Isomerases

Rearrangements in which a hydrogen atom is directly transferred between two adjacent atoms with concomitant exchange of the second substituent, X, which may be a carbon atom with substituents, an oxygen atom of an alcohol, or an amine. These use the adoB_{12} (adenosylcobalamin) form of the vitamin.

2. Methyltransferases

Methyl (-CH_3) group transfers between two molecules. These use MeB_{12} (methylcobalamin) form of the vitamin.

3. Dehalogenases

Reactions in which a halogen atom is removed from an organic molecule. Enzymes in this class have not been identified in humans.

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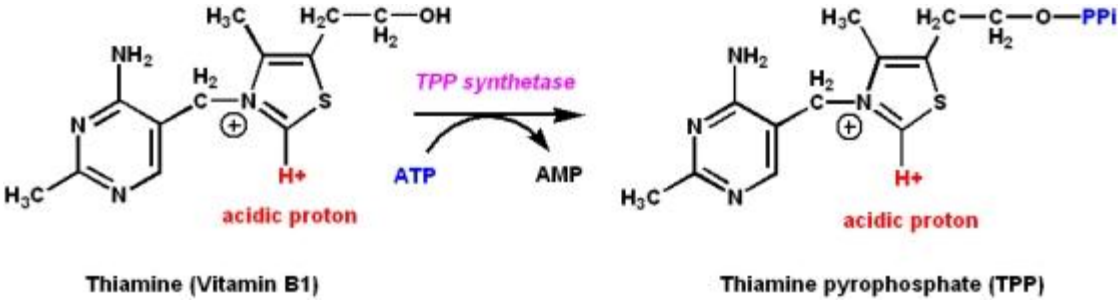
❖ Thiamine pyrophosphate (Vitamin B_1)

Thiamine pyrophosphate (TPP or ThPP), or thiamine diphosphate (ThDP), or cocarboxylase is a thiamine (vitamin B_1) derivative which is produced by the enzyme thiamine diphosphokinase. Thiamine pyrophosphate is a cofactor that is present in all living systems, in which it catalyzes several biochemical reactions. It was first discovered as an essential nutrient (vitamin) in humans through its link with the peripheral nervous system disease Beriberi, which results from a deficiency of thiamine in the diet.

Chemically, TPP consists of a pyrimidine ring which is connected to a thiazole ring, which is in turn connected to a pyrophosphate (diphosphate) functional group.

The part of TPP molecule that is most commonly involved in reactions is the thiazole ring, which contains nitrogen and sulfur. Thus, the thiazole ring is the "reagent portion" of the molecule. The C2 of this ring is capable of acting as an acid by donating its proton and forming a carbanion. Normally, reactions that form carbanions are highly unfavorable, but the positive charge on the tetravalent nitrogen just adjacent to the carbanion stabilizes the negative charge, making the reaction more favorable. (A compound with positive and negative charges on adjacent atoms is called an ylid or ylide, so sometimes the carbanion form of TPP is referred to as the "ylid form".



Thiamine (Vitamin B1) Thiamine pyrophosphate (TPP)

TPP works as a coenzyme in many enzymatic reactions, such as:

- Pyruvate dehydrogenase complex
- Pyruvate decarboxylase in ethanol fermentation
- Alpha-ketoglutarate dehydrogenase complex
- Branched-chain amino acid dehydrogenase complex
- 2-hydroxyphytanoyl-CoA lyase
- Transketolase

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❖ ISOENZYMES OR ISOZYMES

Isozymes (also known as **isoenzymes** or more generally as **Multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage (for example lactate dehydrogenase (LDH)). In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time. Although, strictly speaking, allozymes represent enzymes from different alleles of the same gene, and isozymes represent enzymes from different genes that process or catalyse the same reaction, the two words are usually used interchangeably.

Isozymes or Isoenzymes are proteins with different structure which catalyze the same reaction. Frequently they are oligomers made with different polypeptide chains, so they usually differ in regulatory mechanisms and in kinetic characteristics.

From the physiological point of view, isozymes allow the existence of similar enzymes with different characteristics, "customized" to specific tissue requirements or metabolic conditions.

One example of the advantages of having isoenzymes for adjusting the metabolism to different conditions and/ or in different organs is the following:

Hexokinase, Lactate Dehydrogenase (LDH), Glucokinase, malic dehydrogenase (MDH), esterase and glycol dehydrogenase are examples of isozymes.

In fact, there are four Hexokinases: I, II, III and IV. Hexokinase I is present in all mammalian tissues, and Hexokinase IV, aka Glucokinase, is found mainly in liver, pancreas and brain.

Both enzymes catalyze the phosphorylation of Glucose:

Glucose + ATP $\longrightarrow$ Glucose 6 (P) + ADP

Hexokinase I has a low Km and is inhibited by glucose 6 (P). Glucokinase is not inhibited by Glucose 6 (P) and his Km is high. These two facts indicate that the activity of glucokinase depends on the availability of substrate and not on the demand of the product.

Since Glucokinase is not inhibited by glucose 6 phosphate, in conditions of high concentrations of glucose this enzyme continues phosphorylating glucose, which can be used for glycogen synthesis in liver. Additionally, since Glucokinase has a high Km, its activity does not compromise the supply of glucose to other organs; in other words, if Glucokinase had a low Km, and since it is not inhibited by its product, it would continue converting glucose to glucose 6 phosphate in the liver, making glucose unavailable for other organs (remember that after meals, glucose arrives first to the liver through the portal system).

Uses of isoenzymes in diagnosis

Since isoenzymes have different tissue distributions, their study is an important tool in assessing the damage to specific organs.

Examples of the diagnostic use of isoenzymes are the study of Lactate Dehydrogenase and Creatine Kinase.

Lactate Dehydrogenase (LDH)

It is formed by the association of five peptide chains of two different kinds of monomers: M and H

The variants seen in humans are:

LDH1: M M M M (abundant in heart, brain erythrocytes; around 33% of serum LDH)

LDH2: M M M H (abundant in heart, brain erythrocytes; around 45% of serum LDH)

LDH3: M M H H (abundant in brain, kidneys, lung; around 18 % of serum LDH)

LDH4: M H H H ((abundant in liver, skeletal muscle, kidney; around 3% of serum LDH)

LDH5: H H H H ((abundant in liver, skeletal muscle, ileum; around 1 % of serum LDH)

In myocardial infarction, Total LDH increases, and since heart muscle contains more LDH1 than LDH2, LDH1 becomes greater than LDH2 between 12 and 24 hours, after the infarction, so the ratio LDH1/LDH2 becomes higher than 1 and will stay flipped for several days.

An increase of LDH 5 in serum is seen in different hepatic pathologies: cirrhosis, hepatitis and others. An increase of LDH5 in heart diseases usually indicates secondary congestive liver involvement.

High serum levels of LDH-1 may indicate a tumor of the ovaries or testes, while LDH-5 may indicate liver disease or muscular dystrophy.

Creatine Kinase :

Creatine Kinase (CK) aka Creatine phosphokinase (CPK) is a similar example: three isoenzymes formed by combinations of different subunits:

CK1 (BB) is abundant in brain and smooth muscle (practically absent form serum)

CK2 (MB) is abundant in cardiac muscle, some in skeletal muscle (practically absent from serum)

CK3 (MM) is abundant in skeletal muscle and cardiac muscle (practically 100 % of serum CK)

An elevated serum level of CK-1 indicates a breakdown of skeletal muscle and is one of the signs of muscular dystrophy. An elevated CK-2 level indicates heart disease, because this isoenzyme comes only from cardiac muscle.

- Phosphatasein the blood may indicate bone or prostate disease.
- oxaloacetate transaminase (GOT). In more than 95% of MI patients, serum levels of GOT rise rapidly and then return to normal in 4 - 5 days.

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❖ **Commercial uses of enzymes**

1. Food industry
2. Leather industry
3. Textile industry
4. Environment
5. Phamaceutical and clinical industry

1. Food industry

i. **Acetolactate decarboxylase:** Reduction of maturation time in wine making by converting acetolactate to acetoin.

ii. **Alpha-amylase:** Converts starch to dextrins in producing corn syrup. Solubilizes carbohydrates found in barley and other cereals used in brewing.

iii. **Beta-glucanase:** Breakdown of glucans in malt and and other materials to aid in filtration after mashing in brewing.

iv. **Chymosin:** Curdling of milk by breaking down kappa-caseins in cheese making.

v. **Cellulase:** Conversion of cellulose waste to fermentable feedstock for ethanol or single-cell protein production. Degradation of cell walls of grains, allowing better extraction of cell contents and release of nutrients.

vi. **Glucose oxidase:** Conversion of glucose to gluconic acid to prevent Maillard reaction in products caused by high heat used in dehydration.

vii. **Glucoamylase:** Conversion of dextrins to glucose in the production of corn syrup. Conversion of residual dextrins to fermentable sugar in brewing for the production of "light" beer.

viii. **Lactase:** Additive for dairy products for individuals lacking lactase. Breakdown of lactose in whey products for manufacturing polyactide.

ix. **Lipase:** Enhancing flavor development and shortening the time for cheese ripening. Production of specialty fats with improved qualities. Production of enzyme-modified cheese/butter from cheese curd or butterfat.

x. **Papain:** Used as meat tenderizer. Used in brewing to prevent chill-haze formation by digesting proteins that otherwise react with tannins to form insoluble colloids.

xi. **Pectinase:** Treatment of fruit pulp to facilitate juice extraction and for clarification and filtration of fruit juice.

xii. **Proteases:** Processing of raw plant and animal protein. Production of fish meals, meat extracts, texturized proteins, and meat extenders.

xiii. **Rennet:** Cheese manufacture.

2. Leather industry

i. **Alkaline Proteases:** Used for soaking. When preparing hides and skins for liming and unhairing, proper soaking of the rawstock is essential for obtaining a good quality leather.

ii. **Clarizyme:** Used for dehairing of skins and hides that assists in the removal of hair. It is a unique protease because it is active at the very high pH of 12-13 found in the liming process.Most importantly, enzyme-assisted unhairing results in a cleaner grain surface and improved area yield and softness

iii. **Keratinases:** Hydrolyze the keratin of hair and epidermis

iv. **Lipase:** Used in degreasing of sheep skins. Lipase specifically degrades fat and so cannot damage the leather itself. Lipases hydrolyze not just the fat on the outside of the hides and skins, but also the fat inside the skin structure. Once most of the natural fat has been removed, subsequent chemical treatments such as tanning, re-tanning and dyeing have a better effect.

v. **Proteases:** They hydrolyze the protein fraction of dermatan sulfate, making the collagen. To make the leather pliable, the raw material requires an enzyme treatment before tanning. This is called bating, whereby certain protein components are dissolved and can be washed away. The degree to which bating is applied is dependent on the desired character of the finished leather. Glove leather, for example, should be very soft and pliable and is subjected to strong bating, whereas leather for the soles of shoes is only lightly bated. Leather for shoe uppers falls between these two extremes.

3. Textile industry

Various enzymes like amylases, cellulases, catalase, pectinase and protease are used for various textile wet-processing applications like desizing, bio-polishing, denim finishing, bleach clean-up, bio-scouring and de-wooling.

i. **Amylase**: used to carry out desizing. In the case of fabrics made from cotton or blends of cotton and synthetic fibres, the warp (longitudinal) threads are coated with an adhesive substance known as 'size'. This is to prevent the threads breaking during weaving. The most important size is starch and starch derivatives. After weaving, the size must be removed again in order to prepare the fabric for finishing (bleaching, dyeing, printing etc.). Amylase is a hydrolytic enzyme which catalyses the breakdown of dietary starch to short chain sugars, dextrose and maltose.

ii. **Cellulase:** Used in scouring, the process of removing natural waxes, pectins, fats and other impurities from the surface of fibers, which gives a fabric a high and even wet ability so that it can be bleached and dyed successfully.

iii. **Catalase:** Used in bleaching. Enzymes used for bleach clean-up ensure that residual hydrogen peroxide from the bleaching process is removed efficiently – a small dose of catalase breaks hydrogen peroxide into water and oxygen. This results in cleaner waste water and reduced water consumption.

iv. **Cellulase:** Used for Biofinishing or biopolishing (removing fiber fuzz and pills from fabric surface) – enzymatic biofinishing yields a cleaner surface, softer handfeel, reduces pilling and increases luster. They are also used for Denim finishing. In the traditional stonewashing process, the blue denim was faded by the abrasive action of pumice stones on the garment surface. Nowadays, denim finishers are using a special cellulase. Cellulase works by loosening the indigo dye on the denim in a process known as 'Bio-Stonewashing.

v. Pectinase: It can be used as a bioscour — an environmentally friendly alternative to caustic scouring.

3. **Environment:**

Enzymes are powerful tools that help sustain a clean environment in several ways. They are utilized for environmental purposes in a number of industries including agro-food, oil, animal feed, detergent, pulp and paper, textile, leather, petroleum, and specialty chemical and biochemical industry. Enzymes also help to maintain an unpolluted environment through their use in waste management. Recombinant DNA technology, protein engineering, and rational enzyme design are the emerging areas of research pertaining to environmental applications of enzymes.

i. Chloroperoxidase: catalyzes oxidative chlorination
ii. Ligninolytic enzymes: to degrade recalcitrant environmental pollutants such as aromatic hydrocarbons.
iii. Hydrolases: Break biopolymers gels.
iv. Laccases: oxidation of phenolic compounds
v. Lignin peroxidases: catalyze one-electron oxidations of phenolic and non-phenolic compounds.
vi. Manganese peroxidases: catalyze the Mn-mediated oxidation of lignin and phenolic compounds.

vii. oxidative enzymes: Used in biobleaching and biopulping in the pulp and paper industry, where they can replace environmentally harmful chemicals (e.g. chlorine)

5**. Pharmaceutical and Clinical industry**

i. **Amylase:** Amylases are enzymes that catalyze the hydrolysis of alpha-1, 4-glycosidic linkages of polysaccharides to yield dextrin, oligosaccharides, maltose and D-glucose. It is basically used as a digestive aids.
ii. **Alpha-galactosidase:** It also hydrolyzes indigestible galactans present in intestine and neglects their anti- effect like gas production resulting in bloating, pain and general discomfort.
iii. **Bromelain**: Bromelain is a proteolytic enzyme isolated from the pineapple stem that breaks down other proteins, such as collagen and muscle fiber. Bromelain helps to inhibit pro-inflammatory compounds, similar to non-steroidal anti-inflammatory drugs reducing swelling & pain without side effects. It helps in assisting burn debridement (the removal of dead, burned tissue), preventing blood clots, enhancing fat removal from the body, aiding digestion and reducing sinus symptoms, such as congestion. It is also used for treating arthritis, gout urinary tract infections, ulcerative colitis, chronic obstructive pulmonary disease.
iv. **Cellulase**: Cellulase digests fiber. It helps in the remedy of digestive problems such as malabsorption.
v. **Coenzyme Q10:** Coenzymes help enzymes work to digest food and perform other body processes, and they help protect the heart and skeletal muscles. It also functions as an antioxidant, which protects the body from damage caused by harmful molecules.
vi. **Lactase:** Lactase hydrolyzes disaccharide lactose into constituent monomers, so it is used for treating lactose intolerance disorder in infants and older persons for digestion of lactose.
vii. **Papain, Pepsin, Protease:** These are excellent fibrinolytic and caseionolytic enzymes, which in combination with amylase and lipase prove to be formidable digestive aid. It is also used for preparation of medicines such as ointments for debridement of wounds, inflammatory processes and also for cleaning solutions for soft contact lenses.