Enzymes
Part III: regulation I

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Mechanisms of regulation

- Expression of isoenzymes
- Regulation of enzymatic activity
  - Inhibitors
  - Conformational changes
    - Allostery
  - Modulators
  - Reversible covalent modification
  - Irreversible covalent modification
- Regulation of enzyme amount
- Location (Compartmentalization and complexing of enzymes)
- Non-specific regulation
Isoenzymes (isozymes)

- Isoenzymes are enzymes that can act on the same substrate(s) producing the same product(s).
- They are produced by different genes that vary only slightly.
- Often, various isozymes are present in different tissues of the body.
- They can be regulated differently.
- They can have different catalytic activities.
Lactate dehydrogenases (LDH)

LDH is a tetrameric enzyme composed of a combination of one or two protein subunits: H (heart) and M (skeletal muscle).

These subunits combine in various ways leading to 5 distinct isozymes leading to 5 distinct isozymes (LDH1-5) with different combinations of the M and H subunits.

The all H isozyme is characteristic of that from heart tissue, and the all M isozyme is typically found in skeletal muscle and liver.
The image shows a diagram of LDHB and LDHA subunits and their activities.

- **LDHB** (H, heart) subunit:
  - Nucleus
  - Lactate to Pyruvate conversion
  - NAD\(^+\) to NADH + H\(^+\)

- **LDHA** (M, muscle) subunit:
  - Subunit M
  - Intermediate activity
  - Pyruvate to Lactate reduction
  - NADH + H\(^+\) to NAD\(^+\)

The diagram illustrates the interconversion of lactate and pyruvate, with the involvement of LDH1 to LDH5 enzymes.
Logic behind tissue distribution

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<th>Heart</th>
<th>Kidney</th>
<th>Red blood cell</th>
<th>Brain</th>
<th>Leukocyte</th>
<th>Muscle</th>
<th>Liver</th>
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$$\text{CH}_3\text{C} = \text{COOH} \xrightarrow{\text{LDH}} \text{CH}_3\text{CH} = \text{COOH}$$

Pyruvate \rightarrow Lactate
Muscles can function anaerobically, but heart tissues cannot.

Whereas the all M isozyme (M4) functions anaerobically and catalyzes the oxidation of pyruvate into lactate, the all H enzyme (H4) functions aerobically and catalyzes the reverse reaction.

H4 LDH has low Km for pyruvate and is inhibited by high levels of pyruvate.

M4 LDH has high Km for pyruvate and is not inhibited by pyruvate.
Hexokinase vs glucokinase

Another example is the enzymes hexokinase and glucokinase (Hexokinase IV).

These enzymes catalyze the same reaction converting glucose to glucose-6-phosphate.

However, they are expressed in different tissues and have different kinetic and regulatory properties.

Glucokinase is a liver enzyme, whereas hexokinase is muscle and RBC enzyme.

- The purpose of liver glucose is to balance glucose level in the blood.
- The purpose of muscle and RBC glucose is to produce energy.
Biological significance

It is important to note that once glucose is phosphorylated, it cannot cross plasma membrane out of cells due to the negative charges it carries. Therefore, it is important for liver to have an enzyme with lower efficiency to phosphorylate glucose in order to provide it to other organs like muscles. In contrast, it is important for muscles to have an enzyme with high efficiency in order to trap glucose.
- The Km value of hexokinase for glucose is low (0.1 mM), but it is high for glucokinase (10 mM).
- Hexokinase is inhibited by glucose-6-phosphate, but glucokinase is not.
- Significance: muscles and RBCs do not consume all glucose in blood and liver can convert excess glucose in glycogen for storage.
Regulation of enzymatic activity
inhibitors
Enzyme inhibitors

- Enzyme inhibition can be either reversible or irreversible.
- An irreversible inhibitor dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme, mainly covalently.
  - The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme.
- Reversible inhibition is characterized by a rapid dissociation of the enzyme-inhibitor complex.
  - Usually these inhibitors bind to enzymes by non-covalent forces and the inhibitor maintains a reversible equilibrium with the enzyme.
  - Reversible inhibitors can be competitive or noncompetitive inhibitors.
In competitive inhibition, the inhibitor competes with the substrate for the active site.

Because increasing the amount of substrate can overcome the inhibition, Vmax can be reached in the presence of a competitive inhibitor.

In the presence of a competitive inhibitor, an enzyme will have the same Vmax as in the absence of an inhibitor, but the value of $K_M$ is increased.
In noncompetitive inhibition, the inhibitor binds E or ES complex at a site other than the catalytic site.

Substrate can also bind to the enzyme-inhibitor complex.

However, the enzyme-inhibitor-substrate complex does not proceed to form product.

The value of $V_{\text{max}}$ is decreased while the value of $K_M$ is unchanged.

Noncompetitive inhibition cannot be overcome by increasing the substrate concentration.
Panel A

\[\frac{1}{v} \text{ vs } \frac{1}{[S]}\]

uninhibited enzyme

Panel C

\[\frac{1}{v} \text{ vs } \frac{1}{[S]}\]

\[-\frac{1}{K_m}\]

uninhibited enzyme

Substrate → Enzyme

Substrate

Noncompetitive inhibitor

Enzyme
Mechanism-based inhibitors (irreversible inhibitors)

Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction.

The term includes:
- Covalent inhibitors
- Transition state analogs
- Heavy metals

The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme.
Covalent inhibitors

- Such inhibitors form covalent or extremely tight bonds with active site amino acids.
- Example: DFP is a lethal organophosphorus compound, and a prototype for the nerve gas sarin and the insecticides malathion & parathion.
- DFP inhibits acetylcholinesterase preventing the degradation of the neurotransmitter acetylcholine.

DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal.
Aspirin (acetylsalicylic acid) results in covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase).

Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme
Transition-state analogs: extremely potent inhibitors (bind more tightly)
Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure).
Substrate analogs: even though they bind more tightly than substrates
Also known as suicide inhibitors
Methotrexate is a synthetic inhibitor used to treat cancer.

It is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase, which plays a role in the biosynthesis of nucleotides.

It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis.
Penicillin

- It is a transition-state analog to glycopeptidyl transpeptidase, which is required for synthesis of the bacteria cell wall.
- The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex.
- The active site serine attacks the highly strained β-lactam ring, resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme.
Heavy Metals

- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity.
- Mercury binds to many enzymes, often at reactive sulfhydryl groups in the active site.
  - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity.
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme such as calcium, iron, or zinc by irreversible mechanism.
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca\(^{+2}\) in several regulatory proteins that are important in the central nervous system and other tissues.
Something different: Abzymes

- An antibody that is produced against a transition-state analog & that has catalytic activity similar to that of a naturally occurring enzyme.

- An abzyme is created by injecting a host animal with a transition-state analogue. The host animal makes antibodies to the foreign molecule, & these antibodies have specific binding points that mimic an enzyme surrounding a transition state.
Regulation through conformational changes
These regulatory mechanisms include
- Allostery
- Covalent modulation
- Protein-protein interactions between regulatory & catalytic subunits or between two proteins;
- Proteolytic cleavage

These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation.
Allosteric regulation
Allosteric enzymes

Allosteric enzymes are multi-subunit proteins;
- Multiple active sites that can exist on multiple subunits.
- One subunit containing the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).

The binding of regulatory molecules at sites triggers conformational changes in the active site via modifying non-covalent interactions.
Allosteric modifiers

Allosteric enzymes bind modifiers at the allosteric site, a site that is physically separate from the catalytic site.

- A negative allosteric modifier (inhibitor) causes the enzyme to have less activity.

- A positive allosteric modifier (activator) causes the enzyme to be more active.
More on modifiers

- When the modifier is a molecule other than the substrate, then it is known as heterotropic.
- If the modifier is same as the substrate, then it called homotropic.
- In this case, the binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease.

  This is called "positive cooperativity"
Positive cooperativity

- Allosteric enzymes usually contain two or more subunits and exhibit positive cooperativity where the binding of substrate to one subunit facilitates the binding of substrate to another subunit.
- The first substrate molecule has difficulty in binding to the enzyme because all of the subunits are in the conformation with a low affinity for substrate (the taut “T” conformation) and when it binds, it changes its own subunit and at least one adjacent subunit to the high-affinity conformation (the relaxed “R” state).
The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes.

- The activator ("K effector") generates a hyperbolic curve, with a decrease in the K50 (not Km) and no effect on Vmax since it converts the subunits to the high-affinity state.

- The inhibitor disables the conversion of the subunits to the most active conformation increasing the K50 alone or increasing it with a decrease in the Vmax.

The activator ("K effector") generates a hyperbolic curve, with a decrease in the K50 (not Km) and no effect on Vmax since it converts the subunits to the high-affinity state.
Allosteric enzymes and metabolism

- Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors.
- Allosteric enzymes are not limited to regulation through inhibition and allosteric effectors may function as activators.
- The allosteric effector need not bear any resemblance to substrate or product of the enzyme.
- The effect of an allosteric effector is rapid, occurring as soon as its concentration changes in the cell.
- Feedback regulation of metabolic pathways by end products or by signal molecules that coordinate multiple pathways.
Aspartate transcarbamoylase (ATCase) catalyzes the first step in the synthesis of pyrimidine nucleotides.
ATCase consists of 12 polypeptide chains: six catalytic subunits and six regulatory subunits. It exists in two forms: T state (less active) and R state (more active).
ATCase is inhibited by CTP, the end-product

- inducing a major rearrangement of subunit positions
- stabilizing the T state of the enzyme.
- decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits
- increasing apparent Km
- decreasing enzymatic activity
On the other hand, ATP, a purine, heterotypically activates the enzyme in order to balance the rate of synthesis of purines and pyrimidines in cells.