**ENZYMES: Part II Enzyme Kinetics, Inhibition, and Control**

Enzyme kinetics is the study of rate of reaction of an enzyme catalyzed reaction. Earlier when enzymologist had to work with crude extract of enzyme, one of the approach was to study the rate of reaction and how it changes in response to change in experimental parameters.

* **Effect of substrate concentration on rate of reaction**



Time

Product concentration

The graph shows Initial velocities (Vo) of an enzyme catalyzed reaction. A theoretical enzyme catalyzes the reaction S to P, and is present at a concentration sufficient to catalyze conversion of substrate at maximum velocity V max = min. Here Velocity is rate of reaction. A typical plot of product formed against time for an enzyme-catalyzed reaction shows an initial period of rapid product formation which gives the linear portion of the plot. This is followed by a slowing down of the enzyme rate as substrate is used upand/or as the enzyme loses activity. V0 is obtained by drawing a straight line through the linear part of the curve, starting at the zero time-point. The slope of this straight line is equal to V0.



Initial Velocity

Time

Effect of substrate concentration on initial velocity of a reaction.

At relatively low concentrations of substrate and taking enzyme concentration constant, Vo increases almost linearly with an increase in [S]. At higher substrate concentrations, Vo increases by smaller and smaller amounts in response to increases in [S]. Finally, a point is reached beyond which increases in Vo are small as [S] increases. This plateau like Vo region is close to the maximum velocity, Vmax.

Michaelis and Menten in 1913 postulated that enzyme combines reversibly with its substrate to form an Enzyme Substrate complex in a fast reaction E+S=ES. The ES then breaks down in a slower reaction to free enzyme to form product P. The enzyme thus exist in two form. Free E and Combined ES.

At low [S] the rate of reaction is proportional to [S]. Maximum velocity is observed when all enzymes are present as ES i.e. saturated. This happens when [S] is very high. At this point plateau is observed.

The relation between substrate concentration and rate of reaction can be expressed quantitatively by equation



Where Vo is initial velocity of an enzyme catalysed reaction

Vmax is the maximum velocity

Km is the Michaelis Menten Constant

This is the **Michaelis-Menten** equation, the rate equation for a one-substrate enzyme-catalyzed reaction.

It is a statement of the quantitative relationship between the initial velocity Vo, the maximum velocity Vmax, and the initial substrate concentration [S], all related through the Michaelis constant Km.

When Vo is just half of Vmax i.e. Vo = Vmax / 2

Putting this value in Michaelis-Menten equation and solving we get that

Km=[S] when Vo = Vmax / 2

Thus Km is equivalent to the substrate concentration at which Vo is one-half Vmax

**Effect of Enzyme concentration on rate of a reaction.**

In situations where the substrate concentration is saturating (i.e. all the enzymemolecules are bound to substrate), a doubling of the **enzyme concentration** will lead to a doubling of *V*0. This gives a straight line graph when *V*0 is plotted against enzyme concentration.

**Effect of Temperature on rate of a reaction.**

Temperature affects the rate of enzyme-catalyzed reactions in two ways. First, a rise in temperature increases the thermal energy of the substrate molecules. This raises the proportion of substrate molecules with sufficient energy to overcome the Gibbs free energy of activation (ΔG‡), and hence increases the rate of the reaction. However further increase in temperatureure affect the nature of enzyme. Enzyme gets denatured with increasing temperature.

**Effect of pH on velocity of a reaction.**

Each enzyme has an **optimum pH** at which the rate of the reaction that it catalyzes is at its maximum.

**Enzyme Inhibition**

Any molecule which acts directly on an enzyme to lower its catalytic rate is called an **inhibitor**. Some enzyme inhibitors are normal body metabolites that inhibit a particular enzyme as part of the normal metabolic control of a pathway. Other inhibitors may be foreign substances, such as drugs or toxins, where the effect of enzyme inhibition could be either therapeutic or, at the other extreme, lethal. For example, aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.

There are two broad classes of enzymne inhibitors: reversible and irreversible.

Irreversible Inhibitors- Inhibitors which bind **irreversibly** to an enzyme often form a **covalent bond** toan amino acid residue at or near the active site, and permanently inactivate the enzyme.

A special class of irreversible inhibitors is the suicide inactivators. These compounds are relatively unreactive until they bind to the active site of a specific enzyme. A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted to a very reactive compound that combines irreversibly with the enzyme. These compounds are also called mechanism-based inactivators, because they hijack the normal enzyme reaction mechanism to inactivate the enzyme. Suicide inactivators play a signiflcant role in rational drug design, a modern approach to obtaining new pharmaceutical agents in which chemists synthesize novel substrates based on knowledge of substrates and reaction mechanisms.

The compound **diisopropylphosphofluoridate** (DIPF), a component of nerve gases, reacts with a Ser residue in the active site of the enzyme **acetylcholinesterase**, irreversibly inhibiting the enzyme and preventing the transmission of nerve impulses.

The **antibiotic penicillin** irreversibly inhibits the glycopeptide transpeptidase enzyme that forms the cross-links in the bacterial cell wall by covalently attaching to a Ser residue in the active site of the enzyme.

**Reversible Competitive Inhibitors**

A **competitive inhibitor** typically has close structural similarities to the normal **competitive** substrate for the enzyme. Thus it competes with substrate molecules to bind **inhibition** to the active site. The competitive inhibitor binds **reversibly to the active site**. At **high substrate concentrations** the action of a competitive inhibitor is overcome because a sufficiently high substrate concentration will successfully compete out the inhibitor molecule in binding to the active site. Thus there is no change in the *V*max of the enzyme but the apparent affinity of the enzyme for its substrate decreases in the presence of the competitive inhibitor, and hence *K*m increases.

1. A competitive inhibitor competes with the substrate for the active site of an enzlrne. While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme. Many competitive inhibitors are structurally similar to the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis.

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**Competitive Inhibition**

1. An uncompetitive inhibitor binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex.

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**Regulation of Enzyme activity**

In cellular metabolism,groups of enzyme work together in sequential pathways to carry out a given metabolic process such ast he multireaction break down of glucose to lactate or the multireaction synthesis of an amino acid from simple precursors. In such enzymes systems, the reaction product of y one enzyrne become the substrate of the next. Each pathway has one or more enzymes that have greater effect on overall rate of reaction. Such enzymes are called Regulatory enzyme.

**The activities of regulatory enzymes are modulated in a variety of ways**

**Feedback Regulation-** In biological systems the rates of many enzymes are altered by the presence of other molecules such as activators and inhibitors (collectively known as effectors). A common theme in the control of metabolic pathways is when an enzyme early on in the pathway is inhibited by an end-product of the metabolic pathway in which it is involved. This is called feedback inhibition and often takes place at the committed step in the pathway.

One of the first known examples of allosteric feedback inhibition was the bacterial enzyme system that catalyzes the conversion of L-threonine to L-isoleucine in five steps. In this system, the first enzyme, threonine dehydratase is inhibited by isoleucine, the product of the last reaction of the series. This is an example of heterotropic allosteric inhibition. Isoleucine isquite specific as an inhibitor**.**

**Allosteric enzymes**- In allosteric enzymes, the binding of a substrate molecule to one active site affects the binding of substrate molecules to other active sites in the enzyme. Allosteric enzymes are often multi-subunit proteins, with one or more active sites on each subunit. The binding of substrate at one active site **induces a conformational change** in the protein that is conveyed to the other active sites, altering their affinity for substrate molecules.

In addition, allosteric enzymes may be controlled by **effector** molecules (activators

and inhibitors) that bind to the enzyme at a site other than the active site (either on the same subunit or on a different subunit), thereby causing a change in the conformation of the active site which alters the rate of enzyme activity. Allosteric enzymes function through reversible, noncovalent binding of regulatory compounds called allosteric modulators or allosteric effectors, which are generally small metabolites or cofactors.

Proteolytic activation

Several enzymes are synthesized as larger inactive precursor forms called Zymogens or proenzymes. Zymogens are activated by hydrolysis and breaking away of few peptides. This process is irreversible.

***Pancreatic proteases-*** The digestive enzymes **trypsin**, **chymotrypsin** and **elastase** are

produced as zymogens in the **pancreas**. They are then transported to the small intestine as their zymogen forms and activated there by cleavage of specific peptide bonds. Trypsin is synthesized initially as the zymogen **trypsinogen**. It is cleaved (and hence activated) in the intestine by the enzyme **enteropeptidase** which is only produced in the intestine. Once activated, trypsin can cleave and activate further trypsinogen molecules as well as other zymogens, such as **chymotrypsinogen** and **proelastase.**

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