Types of Enzymes

This Topic presents the most information about enzymes. Make sure you proceed to the Sections on metabolism next, as this is where the greatest application of this knowledge will occur. For human exercise physiology research, the most commonly researched tissue is skeletal muscle, and tissue samples are obtained from the percutaneous biopsy procedure. From such samples, enzymes are indirectly studied by quantifying the rates of the reaction they catalyze. For some enzymes, the procedure is even more indirect, where the product(s) of the reaction are linked to another reaction that is more easily quantified. For enzyme spectrophotometry, most reactions are linked to another reaction that involves an increase or decrease in NADH. When comparing different samples under the same conditions, greater substrate to product conversion is interpreted to mean a greater concentration, or a state of higher activation, of the enzyme for that condition.

Before explaining how some enzymes are controlled, you need to know that not all enzymes are the same. Sure, all enzymes are proteins, are **biological catalysts**, and are highly specific to the substrates of just one reaction. However, enzymes can differ in **substrate affinity**, **reaction velocity**, **pH tolerance**, temperature tolerance, and whether certain other molecules that bind to the enzymes cause **activation** or **inhibition**.

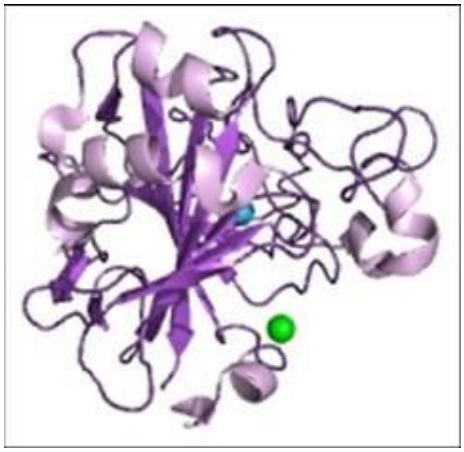

Figure 1. The chemical structure model of carbonic anhydrase. From the Enzyme Commission Protein Database at www.ebi.ac.uk/thornton-srv/databases/enzymes

Figure 2. The chemical structure model of phosphofructokinase. From the Enzyme Commission Protein Database at www.ebi.ac.uk/thornton-srv/databases/enzymes

When you combine these traits with an awareness of the **three dimensional structure** of enzymes, you should be now understanding that enzymes are an incredibly important facet of metabolism and life. Figures 1 to 4 present the three dimensional structure

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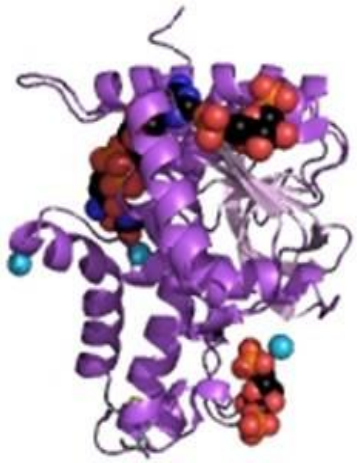

Figure 3. The chemical structure model of lactate dehydrogenase. From the Enzyme Commission Protein Database at www.ebi.ac.uk/thornton-srv/databases/enzymes

Figure 4. The chemical structure model of citrate synthase. From the Enzyme Commission Protein Database at www.ebi.ac.uk/thornton-srv/databases/enzymes

models of **carbonic anhydrase** (blood CO_2 transport), **phoshofructokinase** (glycolysis), **lactate dehydrogenase** (glycolysis) and **citrate synthase** (TCA Cycle). Somewhere within the protein primary, secondary and tertiary structure components, substrates bind to be catalytically converted to products. For many enzymes, other molecules can bind to either activate or inhibit the enzyme, or multiple **substrate binding sites** exist to facilitate improved enzyme function. Thus, enzymes can also be classified by whether they can or cannot be activated or inhibited.

Michaelis-Menten Enzymes

Enzymes can have their catalytic properties revealed by graphing the reaction velocity to substrate concentration (Figure 5). Figure 5 reveals the typical profile for an enzyme obeying **Michaelis-Menten kinetics**, where substrates bind to an enzyme forming an **enzyme-substrate complex**, then proceeding either back to the separate enzyme and substrates or to **product formation**. The mathematic expression of this model is presented in Equation 1.

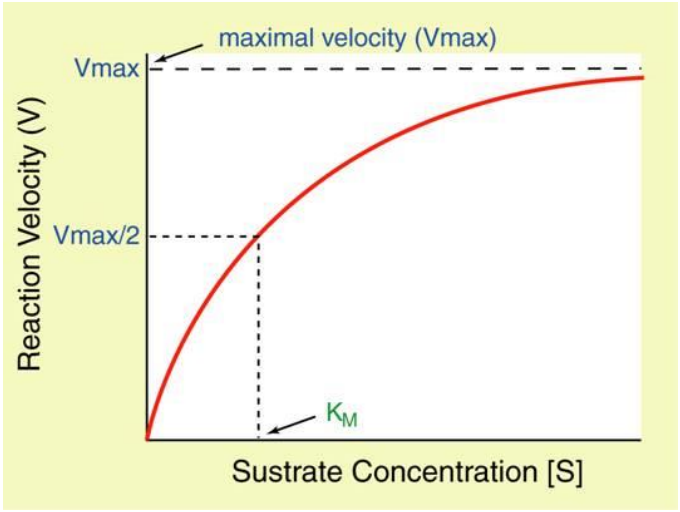

Figure 5. A representative plot of the increase in reaction velocity with increasing substrate concentrations. K_M is the [S] at half Vmax.

$$E + S \underset{\leftarrow k2}{\overset{k1\rightarrow}{\longleftrightarrow}} ES \overset{k3}{\rightarrow} E + P \qquad \text{Equation 1}$$

Note that k_2 is negligible for all **Michaelis-Menten enzyme** catalyzed reactions when [P] is small.

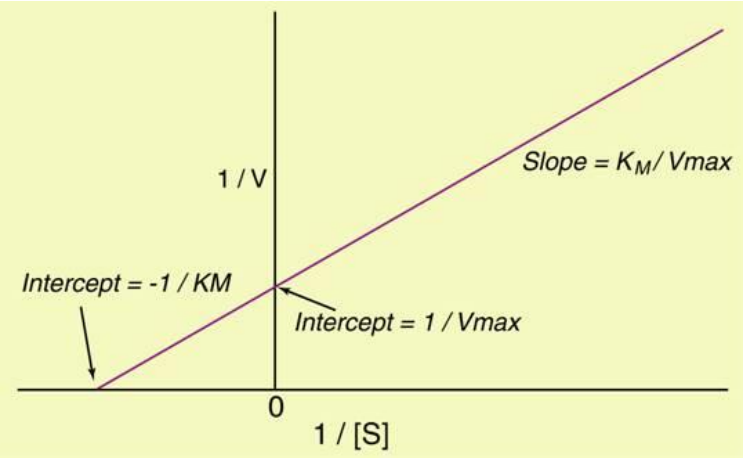

Figure 6. A reciprocal plot, revealing the K_M and Vmax of the enzyme.

Subsequent research of Michaelis-Menten kinetics revealed that from such analyses an enzyme's **maximal catalytic velocity (Vmax)** can be determined, as well as the substrate concentration at half Vmax, referred to as the **Michaelis constant (K_M)**. For example, when data of Figure 5 are reciprocated, and re-graphed (double reciprocal plot) (Figure 6), the Vmax and K_M are easily quantified from the y-intercept and x-intercept, respectively.

As there is never enough substrate inside a cell to tax an enyzme's Vmax capacity, the K_M and corresponding reaction velocity are the most important kinetic properties of an enzyme. The higher the reaction velocity at K_M, and the lower the substrate concentration at K_M, the more effective the enzyme is for catalysis. This is because the enzyme has high rates of catalysis despite a low substrate concentration. Remember, most substrates inside a cell are measured in the mmolar/kg wet wt range.

Michaeilis-Menten enzymes can be inhibited by certain molecules, other than substrates or products, that bind to the enzyme. Such molecules are referred to as inhibitors, and can either bind to the active site, competing with substrates (**competitive inhibitors**), or bind elsewhere on the enzyme while still impairing either of substrate binding (**non-competitive inhibitors**) (Equation 1 *k1*) or the conversion of the ES complex to enzyme plus product (Equation 1 *k2* and *k3*).

Allosteric Enzymes

Not all enzymes operate according to Michaelis-Menten kinetics. Some enzymes have a more sigmoidal V to [S] profile as shown in Figure 7. This different profile exhibits what is called **cooperativity**. This results from the binding of substrate to more than one binding site, or the binding of an activator at a different site (activation), that increases the kinetics of binding between substrates and the enzyme. As such, k1 and k3 from Equation 1 are not constants, but change depending on the nature and extent of substrate and/or activator binding.

A good example of enzyme activation is the adenosine monophosphate (AMP) activation of each of **phosphorylase** (glycogen breakdown) and phosphofructokinase (PFK, glycolysis). As a general rule, remember that allosteric enzymes are typically found near the beginning and end of metabolic pathways. Phosphorylase is important

because it catalyzes the breakdown of **glycogen**, releasing glucose-1-phosphate molecules, which eventually forms **glucose-6-phosphate (G6P)** to fuel glycolysis. PFK catalyzes a reaction near the beginning of **glycolysis**, so these two enzymes, when regulated, control both substrate flux to and through glycolysis. If such reaction locations were not controlled by allosteric enzymes, there would be **glycogenolysis** and glycolysis occurring when the cell did not need such catabolism and free energy release, which would be a waste of energy nutrients. Ideally, the muscle cell, for example, needs to conserve and maximize muscle glycogen for the times it is needed, such as during **intense exercise**. It only makes sense that glycolysis is regulated similarly so that when glycogen is being broken down, glycolysis is able to use these substrate molecules (G6P) and release sufficient free energy to form ATP for muscle contraction.

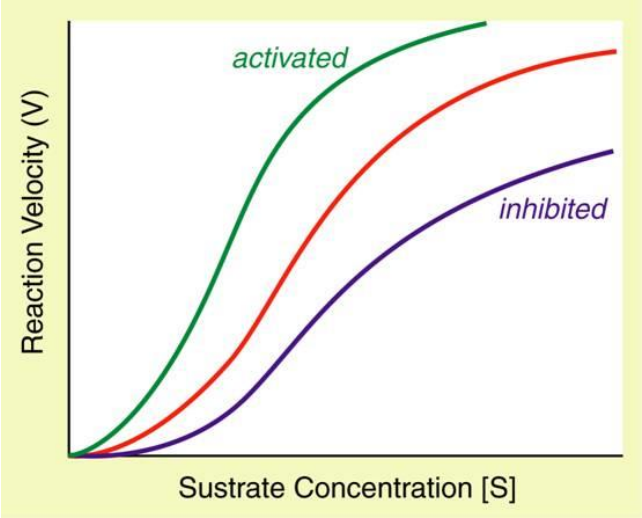

Figure 7. A representative plot of the increase in reaction velocity with increasing substrate concentrations for an allosteric enzyme.

Glossary Words

biological catalysts refers to enzymes, which are proteins that increase the speed of chemical reactions without being a product or substrate, or changing the energetics and therefore directionality of chemical reactions.

substrate affinity is the attraction between an enzyme and the substrate(s) of a specific chemical reaction.

reaction velocity is the rate at which a reaction proceeds to product formation.

pH tolerance when applied to enzymes, refers to the extent that enzyme catalysis is unaffected by changes in pH.

activation refers to the increase in enzyme catalysis in response to the binding of activator compounds/metabolites to the enzyme.

inhibition is the decrease in enzyme catalysis in response to the binding of inhibitor compounds/metabolites to the enzyme.

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three dimensional structure is the result of amino acid chain folding into multiple secondary structures that induce electronic interactions between amino acid side chains resulting in additional conformational alterations.

carbonic anhydrase is the enzyme that catalyzes the conversion of water (H2O) and carbon dioxide (CO2) to carbonic acid (H2CO3), and vice-versa.

phoshofructokinase is the enzyme that catalyzes the conversion of frutsose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6P).

lactate dehydrogenase is the enzyme that catalyzes the conversion of pyruvate to lactate, and vice-versa.

Citrate synthase is the enzyme that catalyzes the conversion of oxaloacetate and acetyl-CoA to citrate, within the TCA cycle of the mitochondria.

substrate binding sites are located within an enzyme 3-dimensional structure where substrate(s) bind via electronic interactions.

Michaelis-Menten kinetics refer the change in an enzyme catalyzed reaction velocity profile with increasing substrate concentrations for enzymes that are not allosterically activated.

enzyme-substrate complex is the complex formed when substrate(s) bind (electronically interact) to an enzyme.

product formation refers to the formation of product in enzyme catalysis.

Michaelis-Menten enzyme is an enzyme that adheres to Michaelis-Menten kinetics, involving a hyperbolic increase in enzyme activity with an increased [S].

maximal catalytic velocity (Vmax) is the maximal rate of catalysis for a specific enzyme. This is a measureable but non-physiological entitiy, as cells never have sufficient substrate for an enzyme to operate anywhere near Vmax.

Michaelis constant (K_M) is the substrate concentration at one-half of Vmax.

competitive inhibitors are compounds/metabolites that when bound (electronically interact) to similar binding sites of an enzyme decrease the rate of enzyme catalysis.

non-competitive inhibitors are compounds/metabolites that when bound (electronically interact) to different binding sites of an enzyme decrease the rate of enzyme catalysis

cooperativity refers to the trait of the reaction velocity to substrate concentration curve (sigmoidal response) resulting from multiple substrate and/or activator binding sites, that

when bound increase the rate of additional binding.

phosphorylase is an enzyme that catalyzes the conversion of glycogen to glucose-1-phosphate (G1P).

glycogen is an intracellular storage molecule of glucose molecules, where glucose molecules are connected end-end and in branched chains.

glucose-6-phosphate (G6P) is the phosphorylated form of glucose when the phosphate is on the sixth carbon.

glycolysis is the pathway of catabolism within the cytosol of a cell responsible for the catabolism of glucose (6carbons) to 2 pyruvate compounds (3 carbons).

glycogenolysis is the breakdown of glycogen to multiple glucose-1-phosphate (G1P) compounds.

Intense exercise is performed at an intensity above the maximal steady state.