BIL 151 Laboratory
Introduction to Biological Catalysts: Enzymes

A **catalyst** is a substance that increases the rate of a chemical reaction without being consumed or permanently changed in the reaction. **Enzymes** are biological **catalysts** that drive specific biochemical reactions. The substance upon which an enzyme operates is its **substrate**.

Protein Structure

Like other proteins, enzymes have

- **primary structure** - the order of amino acids in their polypeptide chains)
- **secondary structure** - coiling or pleating of the polypeptide chain)
- **tertiary structure** – three-dimensional shape formed by spatial relationships of the secondary components
- **quaternary structure** – shape formed by the combination of multiple protein subunits joined to form a single, functional enzyme.

A **domain** is a folded, three-dimensional segment of a **protein** sequence that can evolve and function independently of the rest of the **protein**.
An enzyme's **catalytic domain** forms the **active site**, where its substrate binds.
For the enzyme to be active, the catalytic domain must be folded correctly.

Forces Determining Protein Structure

One of the vital properties of an enzyme is its ability to readily change shape. This property is not only intrinsic to the enzyme, but can be affected by its environment.
Changes in an enzyme's environment can affect its catalytic efficacy and efficiency.

1. Hydrogen bonds

Proton donors (acids) or **acceptors (bases)** may be present in an enzyme's

- amino acid functional groups
- medium/environment

These can affect the active enzyme's

- shape and activity
- affinity for the matrix in which it is embedded

2. Hydrophobic forces

Protein functional groups may be **polar** (**hydrophilic**) or **non-polar** (**hydrophobic**).
In aqueous medium, an enzyme's polar regions may fold inwards, leaving polar regions on the enzyme's surface.

3. Electrostatic forces

Attractions between opposite charges in an enzyme's

- in an enzyme's amino acid functional groups
- between an enzyme and its environment

…can have a strong effect on protein configuration and function.

4. van der Waals forces

These weak repulsive or attractive forces contribute to protein folding.

Enzyme Behavior: The Michaelis-Menten Hypothesis

In 1913, Leonor Michaelis and Maud Menten published a mechanism for the catalysis of chemical reactions in biological systems. Enzyme reaction rate can be expressed with the **Michaelis-Menten equation**:

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

In which:

- $\mathbf{V_o}$ = rate of substrate conversion at a given substrate concentration
- $\mathbf{V_{max}}$ = maximum rate of substrate conversion
- **[S]** = substrate concentration
- $\mathbf{K_M}$ = Michaelis constant

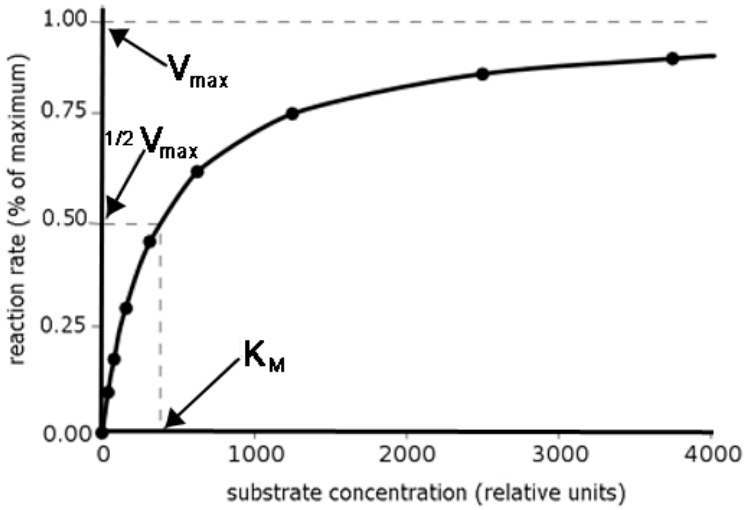

Figure 1. As substrate concentration increases (abscissa), reaction rate increases until the enzyme is completely saturated and working at its maximum possible rate (1.0 on the ordinate).

The **Michaelis constant (K_M)** equals the substrate concentration at which the reaction rate is half of V_{max}. It is an indirect measure of enzyme/substrate affinity (Figure 1).

For any given enzyme/substrate reaction:
- The *lower* the K_M, the *higher* the affinity between enzyme and substrate.
- The *higher* the K_M, the *lower* the affinity between enzyme and substrate.

An enzyme works at maximum speed (V_{max}) when all active sites are occupied by substrate molecules. In this condition, the enzyme is said to be **saturated**. Very high concentrations of substrate are usually needed to reach V_{max}.

To study differences in reaction rate under varied environmental conditions, your team will use a substrate concentration that will allow a measurable rate of reaction, not one at which all substrate is explosively consumed in a few seconds!

Catalase and Hydrogen Peroxide

Peroxidases are a class of enzymes that catalyze the breakdown of peroxide compounds. One of the most ubiquitous and important biological peroxidases is **catalase**, which catabolizes hydrogen peroxide (a toxic byproduct of many metabolic reactions) into harmless water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

As you know, an enzyme's physical and chemical properties are affected by the physical conditions of its environment. Factors such as

- temperature
- pH
- enzyme concentration
- substrate concentration
- various chemicals

…can affect the structure and function of catalase.

If the effect of an environmental variable on catalase structure is known, then changing that variable in a controlled fashion and observing the change in catalase reaction rate can be used to determine whether a particular aspect of enzyme structure is crucial to its function.

Required Reading

Campbell, N. et al. Biology (11th edition) – Chapter 8, pages 153 – 161.

Suggested Reading

Goodsell, D. S. 2004. Catalase. RCSB Protein Data Bank, http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb57_1.html.

Panina, Y., N. Vasyukova, and O. Ozeretskovskaya. 2004. Inhibition of activity of catalase from potato tubers by salicylic and succinic acids. Doklady Biological Sciences 397(1): 131-133.

Seah, TMC and J. G. Kaplan. 1973. Purification and Properties of the Catalase of Bakers' Yeast. Journal of Biological Chemistry 248(8). 2889-2893.

UniProt Consortium 2009. Catalase isozyme 1, *Solanum tuberosum*. The Universal Protein Resource (UniProt), http://www.uniprot.org/uniprot/P49284.