Lab 4: Enzymes

An Enzyme is a catalyst. It speeds up a reaction without being consumed by the reaction. Almost all enzymes are proteins. Recently RNA with enzymatic activities has been discovered. Ribozymes, rRNA in ribosomes, is one example for enzymatically active RNA. Like all catalysts, enzymes lower the activation energy of a reaction ($E_A$ or $\Delta G$) and thus increase the rate of a reaction. The rate of the product formation is measured by the amount of product formed per minute, not the speed of the enzyme.

![Diagram of energy change in a reaction with and without enzyme](image)

Enzymes react with substrates and convert them into different molecules, called products. Almost all processes in a cell need enzymes to facilitate the process. Enzymes are extremely substrate specific. The 3-dimensional structure, due to hydrophilic, hydrophobic, ionic and covalent interactions is crucial for the substrate specificity. Once the enzyme binds to its substrate(s), it will form an enzyme-substrate complex (ES form). The region of the enzyme that binds to the substrate is the active site. This is usually a pocket or a groove on the surface of the enzyme. The enzyme will change its shape due to the substrate binding to its active site. This conformational change is known as induced fit or transition state. The induced fit brings the chemical groups of the active site into positions that enhance their ability to catalyze the reaction. In most cases, the substrate is held in the active site by weak interactions, such as hydrogen bonding and ionic interactions. Side chains of amino acids in the active site catalyze the conversion from a substrate to a product. Once the product is formed, it is released from the active site of the enzyme. The catalytic cycle of an enzyme is illustrated below, using sucrase as an example.
Enzymes can be affected by other molecules. Many vitamins act as coenzymes, minerals act as cofactors for enzymes. Both will activate the enzymatic reaction by binding to the enzyme. Drugs and poisons are often inhibitors of enzymatic activity. Inhibitors may bind to the active site and directly compete with the substrate (competitive inhibitors). An example would be aspirin, which inhibits the COX-1 and COX-2 enzymes involved in producing the inflammatory substance prostaglandin. Other inhibitors may bind to a site apart from the active site but modify the shape of the active site (non-competitive inhibitors). The nerve gas sarin, for instance, is an irreversible inhibitor. Sarin, which was used in the 1995 terrorist attack in the Tokyo subway, is an acetyl cholinesterase inhibitor. Cyanide, also an irreversible inhibitor binds to the enzyme cytochrome C oxidase and inhibits cellular respiration. Many pesticides and antibiotics are also irreversible enzyme inhibitors.

The activity of an enzyme, or how efficiently an enzyme functions, is affected by environmental factors such as temperature and pH. In addition, chemicals may also influence enzyme activity. Each enzyme has an optimal temperature and pH as well as a temperature range and a pH range it might function in. Often, an extreme pH or temperature can denature the enzyme, which may result in sometimes a irreversible inactivation. Imagine, a raw egg being boiled. The egg white changes consistency and color due to the heat. The egg albumin denatures. You cannot convert a boiled egg back into a raw egg. Enzyme denaturation in biological systems is often reversible. Denaturation has to do with the alteration of the three-dimensional structure of the
polypeptide chain. Slight changes in the three-dimensional structure of the enzyme, especially in the active site, will lead to a loss of enzyme activity.

Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. The rate data used in kinetic analyses are obtained from enzyme assays. To find the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is seen. This can be shown in a saturation curve. Saturation happens because, as substrate concentration increases, more and more free enzyme is converted into the substrate-bound ES form. At the maximum velocity ($V_{\text{max}}$) of the enzyme, all the enzyme’s active sites are bound to substrate, and the amount of ES complex is the same as the total amount of enzyme. The Michaelis-Menten constant ($K_m$), is the substrate concentration required for an enzyme to reach one-half its maximum velocity.
In our laboratory exercise, we will study the enzyme catechol oxidase. In the presence of oxygen, catechol (a phenol) is oxidized with the help of catechol oxidase to benzoquinone. Water is formed as a byproduct. Catechol is colorless whereas benzoquinone has a brown color. The enzymatic action can be observed in damaged fruit. Damaged tissue of an apple for instance will turn brown as a result of the action of catechol oxidase. Benzoquinone is antimicrobial, which slows the spoilage of wounded fruits and other plant parts. The production of benzoquinone is thus a protection of the fruit against bacteria.

In the presence of the competitive inhibitor hydroquinone, the enzyme catechol oxidase will bind the hydroquinone at the same speed. But even though hydroquinone fits into the active site (compare the structure of hydroquinone to catechol), the enzyme will not act upon it.

Catechol oxidase needs a cofactor. A cofactor is an inorganic non-protein, usually a metallic ion, that is necessary for the catalytic action of the enzyme. Catechol oxidase needs copper ions (Cu$^{2+}$). PTU (phenylthiourea) is a chelator that binds copper. If all the copper in the solution is bound to PTU, there will be none left to serve as a cofactor for our catechol oxidase. PTU however, is very toxic since many other enzymes in our body need copper as a cofactor. Explain why PTU is toxic.