**Introduction**

We take for granted that with every breath, our lungs will extract oxygen from the air that flows in and eliminate from our bodies the carbon dioxide (CO2) waste produced by cells synthesizing ATP during cellular respiration. CO2 is only minimally soluble in blood plasma, and only a fraction of it can hitch a ride on hemoglobin (Hb) as it cycles back to our lungs. So how does the bulk of the CO2 find its way to exit the body? The answer is the enzyme carbonic anhydrase (CA). CA carries out the following reversible reaction that converts CO2 and H2O into carbonic acid which breaks down into bicarbonate ion (HCO3-) and a hydrogen ion (H+).

CO2 + H2O ⇄ HCO3--+ H+

**A diagram of blood cells

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Figure 1. From top to bottom this figure shows body tissue, blood vessel wall, and red blood cells. CO2 produced in the cells is picked up by the red blood cells where CA converts it to carbonic acid, which breaks down into HCO3- (bicarbonate ion) and a H+. This reaction reverses in the lungs to enable CO2 to be removed from our bodies. When CO2 increases in our bodies, pH tends to decrease, so our breathing rate responds by increasing and releasing CO2 to the atmosphere from our lungs. Thus, CA is essential to both gas transport in the blood and regulating the pH of our bodies.

And it’s not just humans who depend on CA. CA exists in other animals, plants, algae, and bacteria, where it plays some of the same roles. Notably, coral reefs that anchor tropical marine ecosystems are composed of calcium carbonate, which is generated from the bicarbonate produced through CA activity in coral polyps. Algae are especially reliant on CA. CO2 is the starting point for photosynthesis, but just as CO2 has low solubility in our blood, it also has low solubility in water, which is a problem for algae. To resolve this, algae use CA enzyme to concentrate CO2 to levels required for photosynthesis to occur.

**A diagram of a structure

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Figure 2. This figure depicts a chloroplast in an algal or plant cell. Carbonic anhydrase (CA) improves the efficiency of photosynthesis by making larger amounts of CO2 available. CO2 can more readily cross the chloroplast membrane compared to the HCO3-, bicarbonate ion. CO2 that crosses or is transported into the chloroplast is immediately converted into carbonic acid that yields HCO3- and H+ ions. Both ions are used in photosynthesis to fix the carbon in CO2 into the carbohydrates.

As you complete this module you should

* learn more about enzymes by plotting CA enzyme activity under different environmental conditions
* interpret your graph to characterize enzyme kinetics
* consider the roles and effects of inhibitors on enzyme function

**Enzyme Kinetics Exercise**

Two of the most important things to know about an enzyme are the maximal rate at which it can catalyze a reaction, and the amount of substrate at which the enzyme reaches half of that maximum rate. We call the former the Vmax for the reaction, and the latter the Km. These are very useful properties to know because they tell us something about how fast and efficient the enzyme is at catalyzing reactions. The higher the Vmax, the more product an enzyme is capable of generating per unit time under conditions that substrate levels are saturating, and the lower the Km, the less substrate an enzyme requires to reach its half maximum rate.

Data on the amount of product made per unit time, V0 in an enzyme-catalyzed reaction, plotted as a function of the concentration of substrate, [S] can be used to determine the Vmax and the Km for an enzyme. Such a plot can be described by the Michaelis-Menten equation, shown below. The equation is named after the two scientists who first derived the relationship to characterize the Vmax and the Km for enzymatic reactions.

**Michaelis-Menten Equation:**

**A black and white math equations

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**Data analysis/visualization and interpretation**

1. Using the data below for carbonic anhydrase, make a scatter plot with substrate concentration on the x axis and V0 on the y axis.

|  |  |
| --- | --- |
| [S] (mmol/L) | V0 (mmol/s) |
| 0.05 | 2.2 |
| 0.10 | 3.6 |
| 0.20 | 6.7 |
| 0.50 | 14.5 |
| 1.0 | 18.2 |
| 5.0 | 24.0 |
| 10 | 27.7 |
| 15 | 28.4 |

1. Is your plot linear or curved? If curved, does it appear to be linear or nearly linear for any part of the graph, and if so, where? And why do you think the slope changes? That is, thinking about the enzyme and substrate concentration, what about the reaction is causing the slope to decrease when it does?
2. Looking at your plot of [S] vs [V0] for carbonic anhydrase, use what you know about asymptotes, Vmax, and Km to estimate the Vmax and Km for this enzyme.

As you have read in your text, enzymes can be inhibited in multiple ways. Two of the most common types of inhibitors are 1) small molecules that bind the active site because they have a similar molecular shape as the substrate for the enzyme (competitive inhibition), and 2) small molecules that bind to an allosteric site that causes a change in the shape of the enzyme so that the enzyme’s active site still binds substrate but does not function as well (noncompetitive inhibition).

A diagram of different types of site

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Figure 3. Enzyme alone (left), inhibition by a Competitive Inhibitor (middle) and inhibition by a noncompetitive Inhibitor (right)

1. Think about how competitive inhibitors and noncompetitive inhibitors will affect Vmax and Km. Now copy your Michaelis-Menten plot for carbonic anhydrase here, and draw/superimpose on your graph new plots that show/approximate a) how your plot would change in the presence of a competitive inhibitor, and b) how it would change in the presence of a noncompetitive inhibitor. What was the effect of each type of inhibitor on Vmax and Km?
2. As you might recall from the pre-work section of this module, environmental factors like temperature and pH can affect enzyme structure and therefore activity. You measure carbonic anhydrase activity at several different pH values and make a scatter plot of your data. Which of the following plots (a, b, c, d) are you most likely to obtain, and why?

**A graph of different ph values

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