Lab 4 Enzyme Kinetics +

Background

Factors that influence the rate of an enzyme catalyzed reaction.

Amount of enzyme

The measurement of Y_o is proportional to the amount of active enzyme molecules. This feature is important for it enables one to determine the amount of an enzyme in an unknown sample. For example, if 1μg of a pure enzyme gives you a Y_o of 10 nmoles of product/min, a cell extract that yields a Y_o of 20 nmoles product/min contains 2μg of active enzyme.

Temperature

Since enzymes are proteins, they are usually denatured and inactivated by temperatures above 50-70°C. On the other hand, increased temperature also speeds up chemical reactions. With a typical enzyme, the predominant effect of increased temperature up to about 45°C is to increase the enzyme catalyzed reaction rate. Above 45°C, thermal denaturation becomes increasingly important and destroys the catalytic function of an enzyme. At some temperature, a maximum reaction rate called the temperature optimum is observed, and this temperature is usually in the range found in cells (20°C - 40°C).

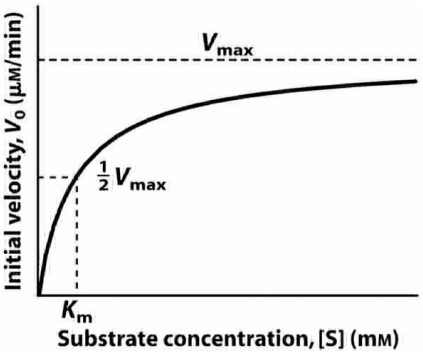
pH

The activity of enzymes is also greatly influenced by acidity or alkalinity. Excess acidity or alkalinity generally causes denaturation and in activation of enzymes, just as high temperature causes heat denaturation. Most enzymes in plants and animal operate effectively at neutral pH. However, the pH optimum of the enzyme that you will study is 4.5. In fact, the name of the enzyme, acid phosphatase, is derived in part from its low pH optimum.

Substrate Concentration

The binding of an enzyme to its substrate is an essential part of the enzyme catalyzed reaction. At low substrate concentration the active site on the enzyme is not working at full capacity. As the concentration of substrate increases, the sites are bound to a greater degree until at saturation, no more sites are available for substrate binding. At this saturating substrate concentration the enzyme is working at full capacity and the maximum velocity (V_{max}) of the reaction is observed.

The consequences of saturating the enzyme with substrate on the reaction rate is seen in a Michaelis-Menton graph. The initial velocity of the reaction (V_o) increases in a hyperbolic manner as the substrate concentration is increased. This increase in reaction rate is proportional to the concentration of the enzyme-substrate complex. Thus, the V_{max} occurs because the enzymes becomes saturated with substrate. The substrate concentration required to yield half the maximal velocity ($V_{max}/2$) can also be determined and is an important constant in describing an enzyme. This constant is known as the Michaelis-Menton constant and is abbreviated K_m. Under conditions of defined temperature, pH and ionic strength, the K_m approximates the dissociation constant of an enzyme for its substrate. The dissociation constant (K_d) of an enzyme is the concentration of substrate that yields half saturation of the enzyme with substrate. Thus the K_d and K_m reflect the affinity of the enzyme for the substrate. For example, a K_m of 0.2mole/l of substrate would indicate that the substrate binding site would be half saturated when the substrate is present at that concentration. Such an enzyme would have a low affinity for its substrate. In contrast, a K_m of 10^{-7} mole/l indicates that the enzyme has a high affinity for its substrate, since it is half saturated at such a low concentration.



Experiment 1 - Determination of V_{max} and K_m

Get 8 testtubes and label 1-8.
Transfer 1 ml of Substrate Dilution Buffer to tubes 1-7.
Transfer 1 ml of Substrate Solution (nitrophenol phosphate (1mM) to tube 8.
Transfer 1 ml from tube 8 to tube 7 and mix.
Transfer 1 ml from tube 7 to tube 6 and mix.
Transfer 1 ml from tube 6 to tube 5 and mix.
Transfer 1 ml from tube 5 to tube 4 and mix.
Transfer 1 ml from tube 4 to tube 3 and mix.
Transfer 1 ml from tube 3 to tube 2 and mix.
Remove 1 ml from tube 2 and discard.

Calculate the substrate concentration for each tube and write the number on the blank line.

	[Substrate]	Abs (410nm)	Product (nmoles)	V_o
Tube 8	1000µM	_____	_____	_____
Tube 7	_____	_____	_____	_____
Tube 6	_____	_____	_____	_____
Tube 5	_____	_____	_____	_____
Tube 4	_____	_____	_____	_____
Tube 3	_____	_____	_____	_____
Tube 2	_____	_____	_____	_____
Tube 1	0 µM	0	0	0

Add 10µl of enzyme (acid phosphatase) to each tube and mix.
Wait 15 minutes
Stop the reaction by adding 1 ml of 1.5% KOH to each tube.
Add 3 ml of water to each tube and mix.

Measure and record the absorbance of each solution at 410 nm.

Using the standard curve you generated in the last laboratory, determine the amount (nmoles) of product (nitrophenol) produced in each tube and record above.

Calculate the V_o (initial velocity) for each reaction. (amount of product/15 min)

Plot your data: V_o on the Y axis and [substrate] on the X axis.
You should get a hyperbolic curve. (It should plateau by the last tube.)
See graph on page 1.
Estimate V_{mac} and K_m

Invert your V_o and [substrate] and make another plot. (Lineweaver-Burke Plot)
$1/V_o$ on the Y axis and 1/[S] on the X axis.
This graph should be a straight line.
Change your X axis so it begins in the negatives so you can find the X intercept.