LS2102 : Lab Assignment 3

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Question 1:

The specific activity is the total moles of product formed by the enzyme over a particular time, under given conditions per total amount of proteins.

$$\text{Specific Activity} = \frac{\text{Enzyme activity}}{\text{Total protein concentration}}$$

The units commonly used for specific activity are katal/Volume(like kat/L), μmol min^{-1} mg^{-1}, nmol min^{-1} mg^{-1} etc

Question 2:

The definitions of Stopped and Continuous assays are as follows :

Stopped Assay : In this assay the reaction is stopped after a fixed time to measure the how much product was formed. The reaction can be stopped by denaturing the enzyme with strong acid, alkali or by heating. For this assay an appropriate stopping time must be chosen such that the velocity time graph is still linear in that range(from start to stopping time). We measure the velocity by taking a slope along the line connecting the initial conditions and the stopping conditions.

Continuous Assay : In this assay we measure activity of the assay continuously. We plot the data obtained and measure the velocity by taking the slop of only the linear part of the curve. Unlike stopped assays we take data during the entire reaction.

Question 3:

The end product is L-Try obtained by hydrolysing L-Phe.

a) The end product L-Tyr is aromatic and will absorb a certain wavelength of light in the UV-region. Tyrosine absorbs a longer wavelength than Phenylalanine. The absorbance wavelength for L-Try is around 280nm and we can use a photo-spectrometer to monitor how much product was formed to calculate its activity.

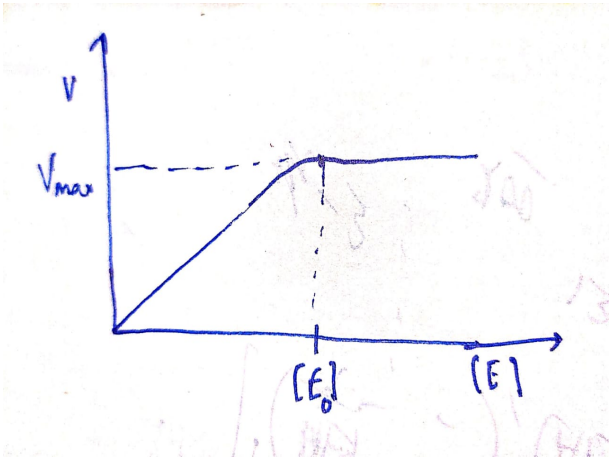
b) K_m is given to be 0.8 mM. The reaction should reach Vmax when we are calculating thus its better to use a Substrate that is higher than K_m. This is done so that no component is rate limiting. Thus any Substrate that is

$$[S]_0 >> K_m$$

is valid thus we can take a value for example 8 - 80mM

c) To obtain the optimum amount of enzyme required we will use the following steps. For a reaction which involves an enzyme we will add a certain small amount of enzyme to the solution. Now we will slowly

keep increasing the enzyme reaction and monitor the activity for each concentration of enzyme. Initially the trend will be linear as shown below in the diagram. Then the trend will become constant. The concentration of the enzyme when the reaction has a maximum velocity is the optimum enzyme concentration($[E]_0$).



Question 4:

For this reaction the substrate Cellobiose is converted to Glucose by an enzyme.
a) The Steps are given as follows

1. First we will calibrating the substrate as a function of absorb.Thus doing this will establish a linear relation between intensity of absorbance and concentration of substrate. Thus for each known values we will measure the absorbance; and plot a Abs(A) vs [Glucose] and fit it with a straight line.

2. Next we will measure the initial reaction velocity(mmol/min)(V) by measuring the final product made in short intervals of time. The concentration of product can be obtained by measuring the absorbance and finding the concentration of the product and substrate using the fitted line.

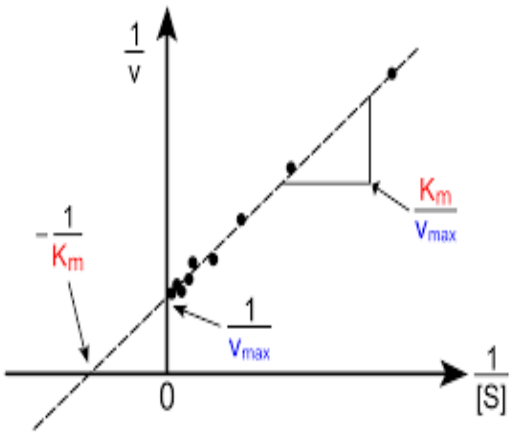
3. We will plot the initial reaction velocity vs substrate concentration on a graph. The graph should be following the michelson meten equation.

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

We can also take the reciprocal of each of those values like so

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]}$$

Now we will plot a 1/V vs 1/[S] plot and fit it to the above equation. To obtain V_{max} and K_M



b) For checking this we must first take substrate at least 10-20 folds higher than the K_M value. This will ensure that the substrate(i.e cellobiose) is high enough so the substrate doesn't act as the limiting agent in the reaction. This will also ensure that there is no backwards reaction. Now we will measure the substrate concentration and activity. Now we will again repeat the experiment only by adding glucose initially. If the L-B plot do not match that means Glucose is an inhibiting agent. WE can conclude weather it is a competitive or noncompetitive inhibitor by comparing the lines to the following graph