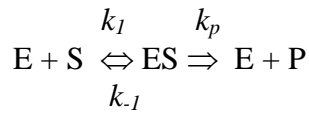


BIOCHEMISTRY 304
Enzyme Kinetic Sample Problems #1
September 2004

1 Given the reaction



where $k_1 = 1 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$, $k_{-1} = 1 \times 10^2 \text{ sec}^{-1}$, and $k_p = 3 \times 10^2 \text{ sec}^{-1}$

- a) Calculate K_s
 b) Calculate K_m

(a)
$$K_s = \frac{k_{-1}}{k_1} = \frac{1 \times 10^2 \text{ sec}^{-1}}{1 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}} = 1 \times 10^{-5} \text{ M}$$

(b)
$$K_m = \frac{k_{-1} + k_p}{k_1} = \frac{(1 \times 10^2 \text{ sec}^{-1}) + (3 \times 10^2 \text{ sec}^{-1})}{1 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}} = 4 \times 10^{-5} \text{ M}$$

2) An enzyme was assayed at an initial substrate concentration of $2 \times 10^{-5} \text{ M}$. In 6 minutes half of the substrate had been consumed. K_m for this enzyme's substrate is $5 \times 10^{-3} \text{ M}$.

- a) Calculate k
 b) Calculate V_{max}
 c) Calculate the concentration of product produced in 15 minutes.

SOLUTIONS

One can by inspection come to the conclusion that this reaction is first-order.

This can be seen as follows:

$$\text{Is } \frac{[S]}{K_m} < 0.01 \quad ? \quad \frac{[2 \times 10^{-5}]}{[5 \times 10^{-3}]} = .004 \quad \text{Yes, first order}$$

a) Since this is a first order reaction we can use the following equation to determine the rate constant, k .

$$\frac{0.693}{k} = t_{1/2} \quad \frac{0.693}{k} = 6 \text{ min} \quad k = \frac{0.693}{6 \text{ min}} \quad k = 0.115 \text{ min}^{-1}$$

$$\text{b) } k = V_{\max} / K_m$$

$$V_{\max} = k \times K_m$$

$$V_{\max} = 0.115 \text{ min}^{-1} \times 5 \times 10^{-3} \text{ M} = 0.575 \times 10^{-3} \text{ M min}^{-1}$$

$$V_{\max} = 0.575 \times 10^{-3} \text{ mole liter}^{-1} \text{ min}^{-1} = 575 \mu\text{moles liter}^{-1} \text{ min}^{-1}$$

c) We know the initial concentration, $[S]_0$, and we have calculated the rate constant, k . We need to determine an arbitrary $[S]$ at 15 minutes after the initial concentration for a first order reaction.

$$2.303 \log \frac{[S]_0}{[S]} = kt$$

$$2.303 \log \frac{[2 \times 10^{-5}]}{[S]} = 0.115 \text{ min}^{-1} \times 15 \text{ min.}$$

$$2.303 \log [2 \times 10^{-5}] - 2.303 \log [S] = 1.725$$

$$2.303 \log [S] = 2.303 \log [2 \times 10^{-5}] - 1.725$$

$$2.303 \log [S] = (2.303 (-4.699)) - 1.725$$

$$2.303 \log [S] = (-10.82) - 1.725$$

$$\log [S] = ((-10.82) - 1.725) / 2.303$$

$$\log [S] = (-12.547) / 2.303$$

$$\log [S] = -5.47$$

$$[S] = 3.56 \times 10^{-6} \text{ M at 15 minutes of reaction}$$

We know that $S_0 = 2 \times 10^{-5} \text{ M}$, the initial concentration

$$[P] = [S]_0 - [S] = 2 \times 10^{-5} \text{ M} - 3.56 \times 10^{-6} \text{ M} = 1.644 \times 10^{-5} \text{ M}$$

3.

An enzyme catalyzes the reaction $S \rightarrow P$. The following data has been obtained. Plot the data to determine K_m and V_{max} using both a Lineweaver-Burke plot and also an Eadie-Hofstee plot.

[S]	v
M	lit-1 min-1
8.33E-06	1.38E-08
1.00E-05	1.60E-08
1.25E-05	1.90E-08
1.67E-05	2.36E-08
2.00E-05	2.67E-08
2.50E-05	3.08E-08
3.33E-05	3.63E-08
4.00E-05	4.00E-08
5.00E-05	4.44E-08
6.00E-05	4.80E-08
8.00E-05	5.34E-08
1.00E-04	5.71E-08
2.00E-04	6.67E-08

SOLUTIONS

Manipulate the given data on the left into forms that can be plotted:

Sample Kinetics Problems 2004

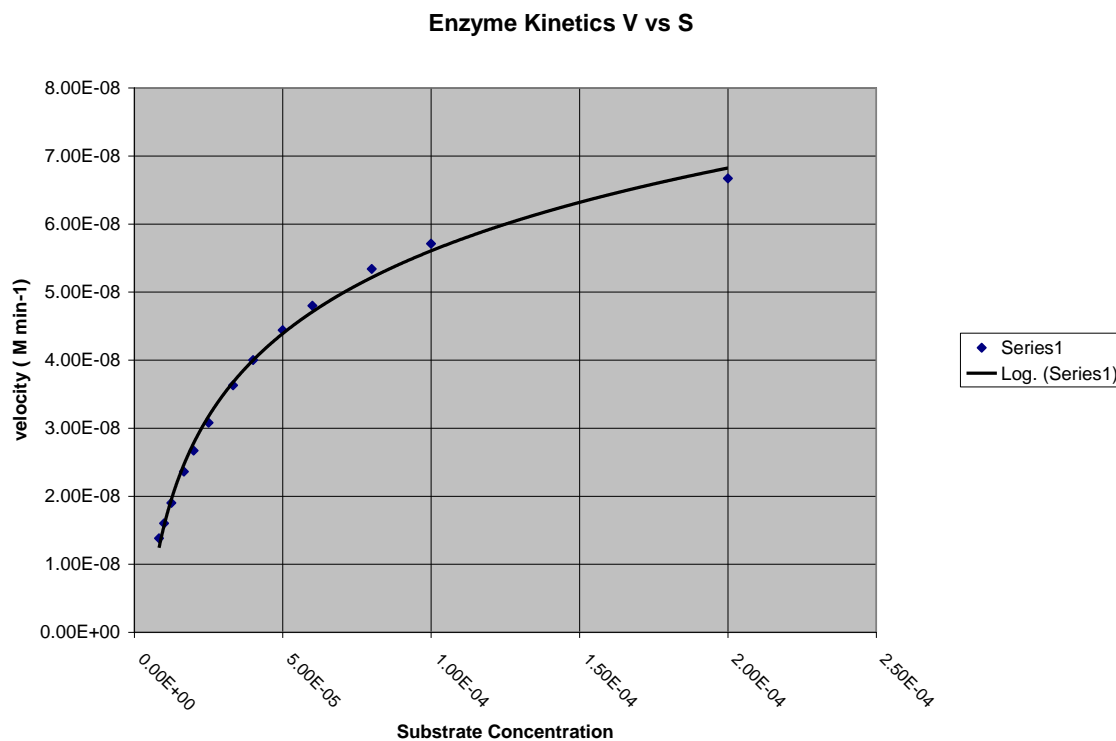
Data for Problem

ORIGINAL DATA		DATA MANIPULATIONS			
[S]	v	1/S	1/v	v/[S]	[S]/v
M	lit-1 min-1	M-1	M -1 min	min-1	min
8.33E-06	1.38E-08	1.20E+05	7.25E+07	1.66E-03	6.04E+02
1.00E-05	1.60E-08	1.00E+05	6.25E+07	1.60E-03	6.25E+02
1.25E-05	1.90E-08	8.00E+04	5.26E+07	1.52E-03	6.58E+02
1.67E-05	2.36E-08	5.99E+04	4.24E+07	1.41E-03	7.08E+02
2.00E-05	2.67E-08	5.00E+04	3.75E+07	1.34E-03	7.49E+02

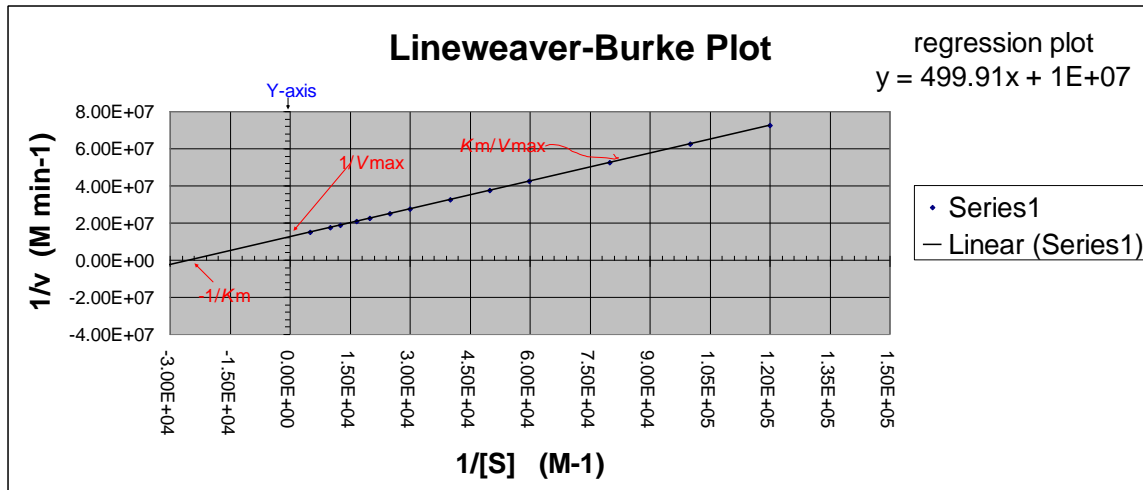
2.50E-05	3.08E-08	4.00E+04	3.25E+07	1.23E-03	8.12E+02
3.33E-05	3.63E-08	3.00E+04	2.75E+07	1.09E-03	9.17E+02
4.00E-05	4.00E-08	2.50E+04	2.50E+07	1.00E-03	1.00E+03
5.00E-05	4.44E-08	2.00E+04	2.25E+07	8.88E-04	1.13E+03
6.00E-05	4.80E-08	1.67E+04	2.08E+07	8.00E-04	1.25E+03
8.00E-05	5.34E-08	1.25E+04	1.87E+07	6.68E-04	1.50E+03
1.00E-04	5.71E-08	1.00E+04	1.75E+07	5.71E-04	1.75E+03
2.00E-04	6.67E-08	5.00E+03	1.50E+07	3.34E-04	3.00E+03

Graphic analysis can be done by plotting the data by hand, using a general program like Microsoft Excel, or a kinetics program. You will need to accurately determine the slope and intercepts in order to extract the kinetic data from hand or Excel plots. A regression line and the regression coefficients would then permit you to calculate the answers numerically.

A simple hyperbolic plot of the velocity versus substrate concentration appears as:



The Lineweaver-Burke plot of $1/v$ vs $1/[S]$ appears as:



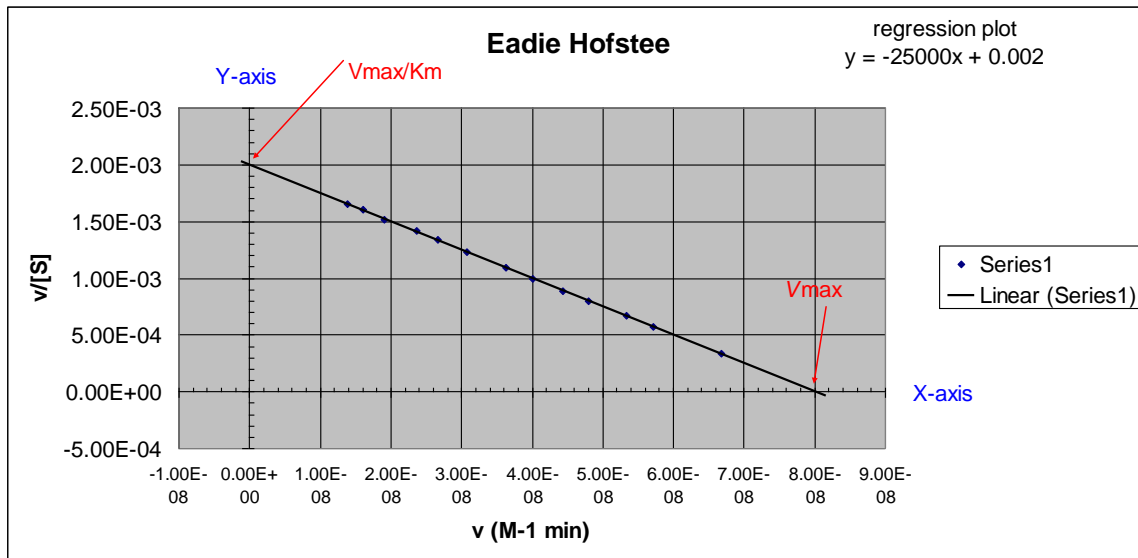
The Y intercept is more precisely: $1.25 * E7 = 1.25 \times 10^7$

The Y intercept = $1/V_{\max} = 1 / 1.25 \times 10^7$
 $V_{\max} = 8.0 \times 10^{-8} \text{ moles liter}^{-1} \text{ min}^{-1}$

The X-intercept = $-2.00E4 = -2.00 \times 10^4$

$K_m = -1/X\text{-intercept} = -1/-(2.00 \times 10^4) = 4 \times 10^{-5} \text{ M}$

The Eadie Hostee plot is done as follows:



The Y intercept is = V_{max}/K_m . The slope is $-1/K_m$. The X intercept is V_{max} .

X intercept	Y intercept	V_{max}/K_m	Slope	K_m
8.00E-08	2.00E-03	2.00E-03	-25000	0.00004

4. Given a crude extract from a cell that contains 20 mg of protein per milliliter of solution. You took ten microliters (10 μ l) of this extract and added it to get a total reaction volume of 0.5 ml. The reaction resulted in the formation of 60 nmoles of product in a time of 1 minute under optimal experimental conditions.

a) Express v as each of the following terms:

- nmoles/assay
- nmoles $\text{ml}^{-1} \text{min}^{-1}$
- nmoles $\text{liter}^{-1} \text{min}^{-1}$
- μ moles $\text{liter}^{-1} \text{min}^{-1}$
- M min^{-1}
- M s^{-1}

b) What would v be in the same 10 μ l of the extract were assayed in a 1.0 ml total reaction volume?

c) What is the enzyme concentration in the assay mixture and on the original extract (expressed as units/ml)?

d) What is the specific activity of the preparation?

SOLUTIONS

a) In order to determine the answer to A one must first write the velocity expression

$$v = 60 \text{ nmoles /assay}$$

this is equal to :

$$v = 60 \text{ nmoles /assay} = \frac{60 \text{ nmoles/min}}{0.5 \text{ ml}}$$

What is the rate in double the volume, i.e 1.0 ml calculate --

$$= 120 \text{ nmoles ml}^{-1} \text{ min}^{-1}$$

What is the rate in 1000 x the volume, calculate --

$$= 120 \times 10^3 \text{ nmoles liter}^{-1} \text{ min}^{-1}$$

What is the rate in umoles vs nmoles (factor of 1,000 again) calculate--

$$= 120 \text{ } \mu\text{moles liter}^{-1} \text{ min}^{-1}$$

What is the rate in M min⁻¹, calculate --

$$= 1.2 \times 10^{-4} \text{ M min}^{-1}$$

What is the rate in M s⁻¹ (60 sec in a minute) calculate—

$$= 2.0 \times 10^{-6} \text{ M s}^{-1}$$

b) This increase in volume would only have the velocity in terms of CONCENTRATION as the SAME amount of product would be formed.

Calculate:

In “a” above we determined ,

$$v = 60 \text{ nmoles} / 0.5 \text{ ml} = 120 \text{ nmoles ml}^{-1} \text{ min}^{-1}$$

but now –

$$v = 60 \text{ nmoles} / 1.0 \text{ ml} = 60 \text{ nmoles ml}^{-1} \text{ min}^{-1}$$

c) Here we calculated before :

$$v = 120 \text{ nmoles ml}^{-1} \text{ min}^{-1} = 0.120 \text{ } \mu\text{moles ml}^{-1} \text{ min}^{-1}$$

This is equal to 0.12 units/ ml of the assay mixture

$$[E]_t = 0.12 \text{ units/ ml assay mixture}$$

However the actual (original) assay was a volume of 0.5 ml thus it contained only 0.06 units and we used only 10 μl (0.01 ml) of the extract to add to the original reaction.

Therefore we can calculate

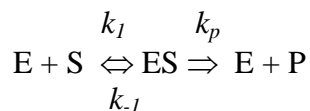
$$[E]_t = \frac{0.06 \text{ units}}{0.01 \text{ ml}} = 6 \text{ units / ml extract}$$

d) The specific activity is calculated by the amount of enzyme. This is usually in terms of per mg protein since enzymes are proteins. The pure the extract the higher the specific activity since more of the protein measured is actually the enzyme.

$$\text{Specific activity} = \text{SA} = \frac{6 \text{ units / ml}}{20 \text{ mg protein / ml}}$$

$$\text{SA} = 0.30 \text{ units / mg protein}$$

5. Given the reaction of an enzyme that follows Michaelis-Menten kinetics:



$$\text{If } K_m = 30 \text{ mM and } V_{\max} = 60 \text{ } \mu\text{M min}^{-1}$$

- What is the initial reaction velocity at a substrate concentration of 0.1 mM?
- What is the initial reaction velocity at a substrate concentration of 30 mM?
- What is the initial reaction velocity at a substrate concentration of 1000 mM?

SOLUTIONS

This is a basically statement of the Michaelis-Menten analysis, therefore, the relevant equation is

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

a)

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

$$V_0 = \frac{60 \mu\text{M min}^{-1} [100 \mu\text{M}]}{30000 \mu\text{M} + [100 \mu\text{M}]}$$

$$V_0 = \frac{6000 \mu\text{M}^2 \text{min}^{-1}}{30100 \mu\text{M}}$$

$$V_0 = 0.199 \mu\text{M min}^{-1}$$

b)

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

$$V_0 = \frac{60 \mu\text{M min}^{-1} [30000 \mu\text{M}]}{30000 \mu\text{M} + [30000 \mu\text{M}]}$$

$$V_0 = \frac{1.8 \cdot 10^6 \mu\text{M}^2 \text{min}^{-1}}{60000 \mu\text{M}}$$

$$V_0 = 30 \mu\text{M min}^{-1}$$

c)

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

$$V_0 = \frac{0.060 \text{ mM min}^{-1} [1000 \text{ mM}]}{30 \text{ mM} + [1000 \text{ mM}]}$$

$$V_0 = \frac{60 \text{ mM}^2 \text{min}^{-1}}{1030 \text{ mM}}$$

$$V_0 = 5.83 \times 10^{-2} \text{ mM min}^{-1} = 58.3 \mu\text{M min}^{-1}$$