Analysis of Enzyme Kinetics in Invertebrates

- Derivation of the Michaelis-Menten Relation
- Experimental Use of the Michaelis-Menten Equation
- Enzyme Kinetics Experiment

Derivation of the Michaelis-Menten Relation

I. Background

A. The M-M relation describes how the rate of a "reaction" depends on the amount of a "substance" that is transformed.

B. Classical example: enzyme and substrate.

The transformed substance is sucrose. The reaction is the creation of glucose and fructose from sucrose.

C. "Reaction" and "substrate" need not be chemical

II. Chemical Equations

Two compounds (A and B) combine at rate k_1 to form compound C. Compound C dissociates at rate k_2. What is the chemical equation?

III. Mathematical representation of chemical dynamics

A. Convert to **rates of change** for A, B, and C

B. Integrate rates to get amounts of A, B, and C at different times

C.

$$\frac{dA}{dt} = -k_1AB + k_2C$$

$$\frac{dB}{dt} = -k_1AB + k_2C$$

$$\frac{dC}{dt} = k_1AB - k_2C$$

D. Steady-state (equilibrium) of the system:
All of the above rates equal 0

E. Mass Action: random "encounter" of compounds A and B is proportional to the product of concentrations of A and B

F. Stoichiometry:

$$\underbrace{2A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C}_{\text{chemical}} \Longrightarrow \underbrace{\frac{dC}{dt} = k_1 A^2 B - k_2 C}_{\text{mathematical}} \qquad (1)$$

IV. Enzyme Kinetics and Michaelis-Menten

A. Chemical equation

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \overset{k_3}{\rightharpoonup} E + P$$

where E=enzyme, S=substrate, C=complex, and P=product.

B. Mathematical Equation

How many rate equations? What symbols? What equations?

C. Simplify: Apply Conservation of Mass

let $r = E + C$ = total number of molecules of E in a unit volume

so, $E = r - C$

This means we don't need an explicit rate equation for E.

New equations after substituting equation for E:

$$\frac{dS}{dt} = -k_1 S(r - C) + k_2 C$$

$$\frac{dC}{dt} = k_1 S(r - C) - (k_2 + k_3)C$$

$$\frac{dP}{dt} = k_3 C$$

But, dS/dt and dC/dt do not depend on P, so P can be solved later once we know C.

D. Simplify: Quasi-steady-state for C

1. Assume

$$\frac{dC}{dt} \approx 0$$

This is a quasi-steady-state, since $dS/dt \neq 0$. We are assuming that the intervals between observations is long enough that C reaches steady-state between observations.

2. Solve for C at quasi-steady-state

$$0 = k_1 r S - (k_1 S + (k_2 + k_3))C$$

implies

$$C = \frac{k_1 r S}{(k_2 + k_3) + k_1 S}$$

3. Re-write S rate equation

$$\begin{aligned} \frac{dS}{dt} &= -k_1 r S + k_1 S C + k_2 C \\ &= \boxed{-k_1 S(r - C)} + k_2 C \\ &= \boxed{-(k_2 + k_3)C} + k_2 C \\ &= -k_3 C \end{aligned}$$

Notice the substitution of the boxed material. Why are these two quantities equal?

4. Subsitute C in dS/dt

$$\begin{aligned} \frac{dS}{dt} &= -k_3 \left[\frac{k_1 r S}{(k_2 + k_3) + k_1 S} \right] \\ &= -\frac{k_3 r S}{\left(\frac{k_2 + k_3}{k_1}\right) + S} \end{aligned}$$

E. What about P, the product?

1.

$$\frac{dP}{dt} = k_3 C = -\frac{dS}{dt} = \frac{k_3 r S}{\left(\frac{k_2+k_3}{k_1}\right) + S}$$

2. What is the shape of the curve relating dP/dt and S?

3. Strip away complicated parameters:

$$\frac{dP}{dt} = v \boxed{\frac{S}{u+S}}$$

Call the box W. What is the graph of W? Cases to consider:

i. If $S = 0$, then $\boxed{W = \quad}$

ii. If $S << u$, $\boxed{W = \quad}$

iii. If $S >> u$, $\boxed{W = \quad}$

Sketch the graph of dP/dt in the axes below.

F. Interpret the constants biochemically.

1. $v = k_3 r$. In words, v is the maximum rate of product (P) formation. Call it $V_{\max}$, (V for "velocity").
   What are the units of $V_{\max}$?

Units of $V_{\max}$ =

2. $$u = \frac{k_2 + k_3}{k_1}$$

   So far, we have:

   $$\frac{dP}{dt} = \frac{V_{\max} S}{u + S}$$

   What are the units of u?

Units of u =

   Do the overall units of dP/dt check out?
   To give an intuitive interpretation of u, we will use a CMT (**C**lever **M**athematical **T**rick) and re-arrange the equation above for dP/dt (algebra steps left for student):

   $$\frac{u}{S} = \frac{V_{\max} - dP/dt}{dP/dt}$$

Suppose $S = u$, then $u/S = 1$ and

$$\frac{dP}{dt} = V_{\max} - dP/dt$$

$$\text{or} \qquad 2\frac{dP}{dt} = V_{\max}$$

$$\text{or} \qquad \frac{dP}{dt} = \frac{1}{2}V_{\max}$$

In words, when $S = u$, the rate of product formation is *one-half the maximum rate of product formation.* So, u is the $\frac{1}{2}$*-saturation constant.* Also known as the *Michaelis-Menten constant* $= K_m$.

G. Finally, we're done. The Michaelis-Menten equation that relates the rate of product formation to the substrate concentration is:

$$\frac{dP}{dt} = \frac{V_{\max} S}{K_m + S}$$

And has the graph: