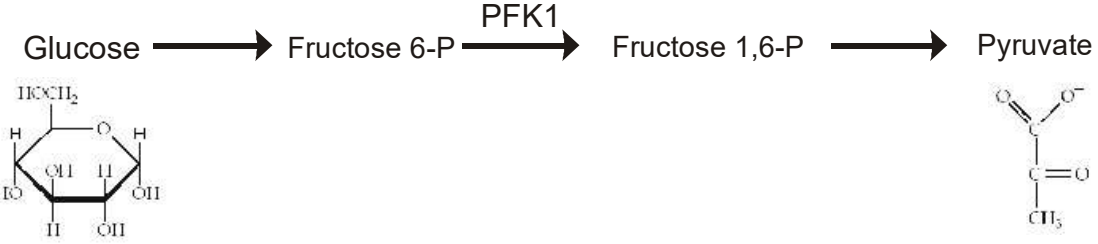
**Quantitative Physiology I / Molecular and Cellular Systems, BMEN E4001x**
**Notes 02 - Enzyme kinetics**
**Chapter 1 of Keener & Sneyd**

# Life is based on regulation of processes (see B&B Chapt. 58)

Glycolysis is a key step in carbohydrate metabolism. This is the breakdown of glucose into pyruvate, which goes into the citric acid cycle, yielding two energy stores, ATP and NADH. Here are the first few steps, for which enzyme regulation plays a key role.




This process takes some ATP, converting it to the lower energy ADP, but ATP is replenished later in metabolism. In this scheme, consider phosphofructokinase (PFK1)

- adds a second phosphate group (from ATP) to fructose, leading later to splitting of this molecule
- ATP is a substrate for this enzyme
- ATP is also an inhibitor of PFK1, leading to feedback regulation of this enzyme and metabolism

# Enzyme Kinetics

In an enzymatic relation, something happens after the binding. So, our equations need to include this conversion. However, measured kinetics show a slightly different kinetics than would be alluded to using our bimolecular model.

Note that for the initial A+B->C reaction, the initial rate reaction, assuming [C](t=0)=0 is:

$$\frac{dc}{dt} = abk_1 \Rightarrow \frac{d[C]}{dt} = [A][B]k_1$$

In contrast, enzymatic systems often show saturation.

A simple mechanism to express simple enzyme kinetics, Michaelis Menten (1913):

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$$

This system allows regeneration of E.
It is also recognized that P+E-> C can conceptually happen, but it is assumed here that P gets removed immediately.

$$\frac{ds}{dt} = -sek_1 + ck_{-1}$$
$$\frac{de}{dt} = -sek_1 + ck_{-1} + ck_2$$
$$\frac{dc}{dt} = +sek_1 - ck_{-1} - ck_2$$
$$\frac{dp}{dt} = ck_2$$
$$e + c = e_0$$

## *Equilibrium Approximation*

In this *original* form, Michaelis and Menten assumed fast equilibrium between the S,E and C. Thus:

$$k_1se=k_{-1}c$$

and by using $e+c=e_0$, with a similar chain of algebra as for biomolecular binding.

$$k_1s(e_0-c)=k_{-1}c$$
$$se_0-sc=(k_{-1}/k_1)c$$
$$c[(k_{-1}/k_1)+s]=se_0$$
$$c=e_0*s/((k_{-1}/k_1)+s)$$

At the end, they got:

$$c = e_0 \frac{s}{K_s + s}; [C] = [E]_0 \frac{[S]}{K_s + [S]}; K_s = \frac{k_{-1}}{k_1}$$

Quite reasonable, when it is recognized that this is the same form as bimolecular binding. Put another way, this is bimolecular binding leading to a slow evolution of C into P + E. As the next step is

$$\frac{d[P]}{dt} = k_2[C]$$

we can say that the rate of reaction is proportional to [C], or

$$V = k_2[E]_0 \frac{[S]}{K_S + [S]}; K_S = \frac{k_{-1}}{k_1}$$

## *Quasi-steady-state approximation*

Briggs and Haldane (1925) proposed a refinement, one in that the rate constants associated with production and use of intermediary species are equal. That is, for an intermediary species such as [C], d[C]/dt is small during the bulk of the reaction following an initial, high excursion period.

Formally, make the following non-dimensionalization substitutions:

$$\sigma = \frac{s}{s_0}; \quad \chi = \frac{c}{e_0}; \quad \tau = k_1 e_0 t; \quad \kappa = \frac{k_{-1} + k_2}{k_1 s_0}; \quad \varepsilon = \frac{e_0}{s_0}; \quad \alpha = \frac{k_{-1}}{k_1 s_0}$$

In non-dimensionalization, these values are plugged back into the original system of equations. Here, the important one is to look at the dc/dt equation.

$$\frac{d\chi}{d\tau} = \frac{dc}{dt}\left(\frac{d\chi}{dc}\right)\left(\frac{dt}{d\tau}\right) = \frac{1}{k_1 e_o^2}\frac{dc}{dt}$$

$$\frac{dc}{dt} = +se_0k_1 - csk_1 - ck_{-1} - ck_2; (\text{using } e = e_0 - c)$$

$$\frac{dc}{dt} = +se_0k_1 - \left[c(sk_1 + k_{-1} + k_2) \xrightarrow{\text{pull out } k_1 s_0} ck_1 s_0\left(\frac{s}{s_0} + \left(\frac{k_{-1} + k_2}{k_1 s_0}\right)\right)\right]$$

now, making the non - dimensionalized equivalents as possible

$$\frac{dc}{dt} = +\sigma * s_0 * e_0 * k_1 - c * k_1 * s_0 * (\sigma + \kappa)$$

and, multiplying by the terms to get to the nondimensional differential

$$\frac{d\chi}{d\tau} = \left(\sigma\left(\frac{s_0}{e_0}\right) - \frac{c}{e_0}\left(\frac{s_0}{e_0}\right)(\sigma + \kappa)\right)$$

$$\frac{d\chi}{d\tau} = \frac{1}{\varepsilon}(\sigma - \chi(\sigma + \kappa))$$

Now, enzymes are very effective, so a little acts on a lot of enzyme with high efficiency. Thus, ε is typically small. Thus, dχ/dτ is very responsive to changes in the other variables, adjusting to them. Moreover, this acts to restore χ towards some equilibrium value. So, we can approximate dχ/dτ ~ 0.

Working this through dc/dt=0 leads to:

$$V = k_2[E]_0 \frac{[S]}{K_M + [S]}; K_M = \frac{k_{-1} + k_2}{k_1}$$

Equations of these forms are generally referred to as Michaelis-Menton forms; the switch from $K_S$ to $K_M$ is reflective of this change.

# Regulation of Enzyme Kinetics

## *Competitive Inhibition*

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} P + E$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2$$

with system of equations

$$\frac{ds}{dt} = -k_1 se + k_{-1} c_1$$

$$\frac{de}{dt} = -k_1 se + k_{-1} c_1 + k_2 c_1 - k_3 ie + k_{-3} c_2$$

$$\frac{di}{dt} = -k_3 ie + k_{-3} c_2$$

$$\frac{dc_1}{dt} = k_1 se - (k_{-1} + k_2) c_1$$

$$\frac{dc_2}{dt} = k_3 ie - k_{-3} c_2$$

$$\frac{dp}{dt} = k_2 c_1$$

$$e + c_1 + c_2 = e_0$$

Use the quasi-steady state approximation for $C_1$ and $C_2$

$C_1$:

$$k_1 s(e_0 - c_1 - c_2) - (k_{-1} + k_2) c_1 = 0$$

$$s(e_0 - c_1 - c_2) - K_M c_1 = 0; \;\; K_M = \frac{(k_{-1} + k_2)}{k_1}$$

$$s(e_0 - c_1 - c_2) = K_M c_1; \;\; K_M = \frac{(k_{-1} + k_2)}{k_1}$$

$C_2$:

$$k_3 i(e_0 - c_1 - c_2) = k_{-3} c_2$$

$$k_3 i(e_0 - c_1) = k_3 i c_2 + k_{-3} c_2$$

$$i(e_0 - c_1) = c_2 (i + K_i); \;\; K_i = \frac{k_{-3}}{k_3}$$

$$\Rightarrow c_2 = \frac{i(e_0 - c_1)}{i + K_i} = \frac{e_0 - c_1}{(1 + K_i / i)}$$

plug expression for $C_2$ back into eqn. for $C_1$

$$se_0 - sc_1 - s\frac{(e_0 - c_1)}{(1 + K_i/i)} - K_M c_1 = 0$$

$$se_0 - s\frac{(e_0 - c_1)}{(1 + K_i/i)} - (s + K_M)c_1 = 0$$

Multiply by $(1+K_i/i)$, change signs

$$-se_0(1 + K_i/i) + se_0 - sc_1 + (s + K_M)(1 + K_i/i)c_1 = 0$$

expand, group $c_1$'s on left, $e_0$'s on right

$$c_1\left[-\mathbf{s} + \mathbf{s} + s(K_i/i) + K_M + K_M K_i/i\right] = se_0(\mathbf{1} + K_i/i - \mathbf{1})$$

canceling out symbols in bold, solving for $c_1$

$$c_1 = \frac{se_0(K_i/i)}{s(K_i/i) + K_M + K_M K_i/i}$$

rearrange into

$$c_1 = \frac{se_0}{s + K_M(1 + i/K_i)}$$

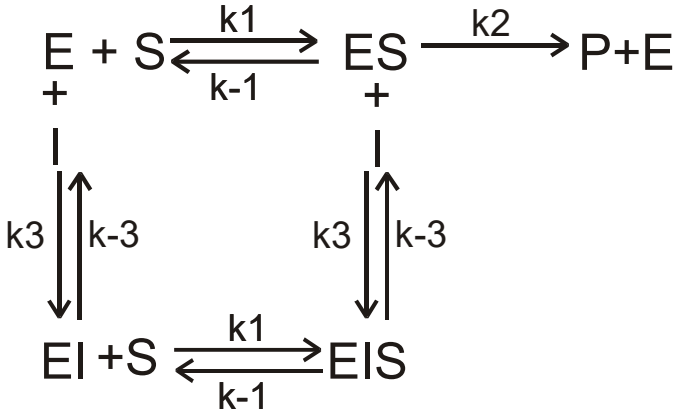
This gives us

$$V = k_2 c_1 = k_2[E]_0 \frac{[S]}{K_M(1 + [I]/K_i) + [S]} = V_{max} \frac{[S]}{K_M(1 + [I]/K_i) + [S]}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}; K_i = \frac{k_{-3}}{k_3}$$

## *Non-competitive Allosteric Inhibition*

Allosteric site binds to E or ES, preventing conversion of ES to P+E




Following as Keener & Sneyd work through the equilibrium case, which was derived from rapid equilibrium over the four double-headed arrows, with the convention of $e=e_0-x-y-z$, where

X=ES Y=EI Z=EIS

1. $k_1se=k_{-1}x$
2. $k_3ie=k_{-3}y$
3. $k_1sy = k_{-1}z$
4. $k_3ix = k_{-3}z$

This is a system of four equilibria. Note that by going around a box and using transitivity, one of these equations is not needed.

The full derivation is included below. Let's skip this for class, and jump to the result, indicated a few lines below.

Solve eqn 1 for x

$$-x(k_1s + k_{-1}) + k_1s(e_0 - y - z) = 0$$

Use eqn 3 ($y=zk_{-1}/(k_1s)$)

$$x(k_1s + k_{-1}) = k_1s(e_0 - z(1 + k_{-1}/(k_1s))$$

Use eqn 4 ($z=xik_3/k_3$)

$$x(k_1s + k_{-1}) = k_1s\left(e_0 - xi\left(\frac{k_3}{k_{-3}}\right)\left(1 + \frac{k_{-1}}{k_1s}\right)\right)$$

Mult. both sides by $k_{-3}/k_3$ and also divide both sides by $k_1$

$$x(s + \frac{k_{-1}}{k_1})\left(\frac{k_{-3}}{k_3}\right) = s\left(e_0\left(\frac{k_{-3}}{k_3}\right) - xi\left(1 + \frac{k_{-1}}{k_1s}\right)\right)$$

Now, using

$K_i=k_{-3}/k_3$ and $K_s=k_{-1}/k_1$

we get

$$x(s + K_s)K_i = s\left(e_0K_i - xi\left(1 + \frac{K_s}{s}\right)\right) = se_0K_i - xi(s + K_s)$$

rewrite as

$$x(s + K_s)(K_i + i) = se_0K_i$$

**AND FINALLY**

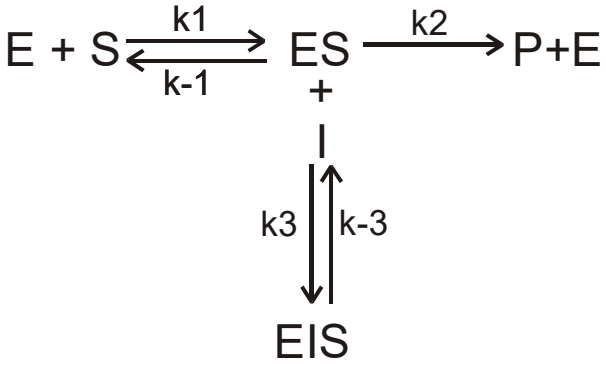
$$x = \frac{se_0K_i}{(s + K_s)(K_i + i)}$$ ; $K_i=k_{-3}/k_3$ and $K_s=k_{-1}/k_1$

and in terms of rates

$$V = k_2 \frac{se_0K_i}{(s + K_s)(K_i + i)} = V_{max} \frac{1}{1 + [I]/K_i} \frac{[S]}{K_s + [S]}$$

## *Uncompetitive Allosteric Inhibition*

Allosteric site binds to ES (but not E), preventing conversion of ES to P+E




X=ES Y=EIS

Equilibrium approach, system equations become:

1. $k_1es = k_{-1}x$
2. $k_3ix = k_{-3}y$
3. $e_0 = e + x + y$

Rearrange 2 into

$$y = \frac{k_3}{k_{-3}}xi = K_i^{-1}xi;\ K_i = \frac{k_{-3}}{k_3}$$

Combine equations 1 & 3

$$k_1s(e_0 - x - y) = k_{-1}x$$
$$x(k_{-1} + k_1s) + k_1sy = k_1se_0$$

Substitute for y and rearrange

$$x\left(k_{-1} + k_1 s + k_1 s i K_i^{-1}\right) = k_1 s e_0$$

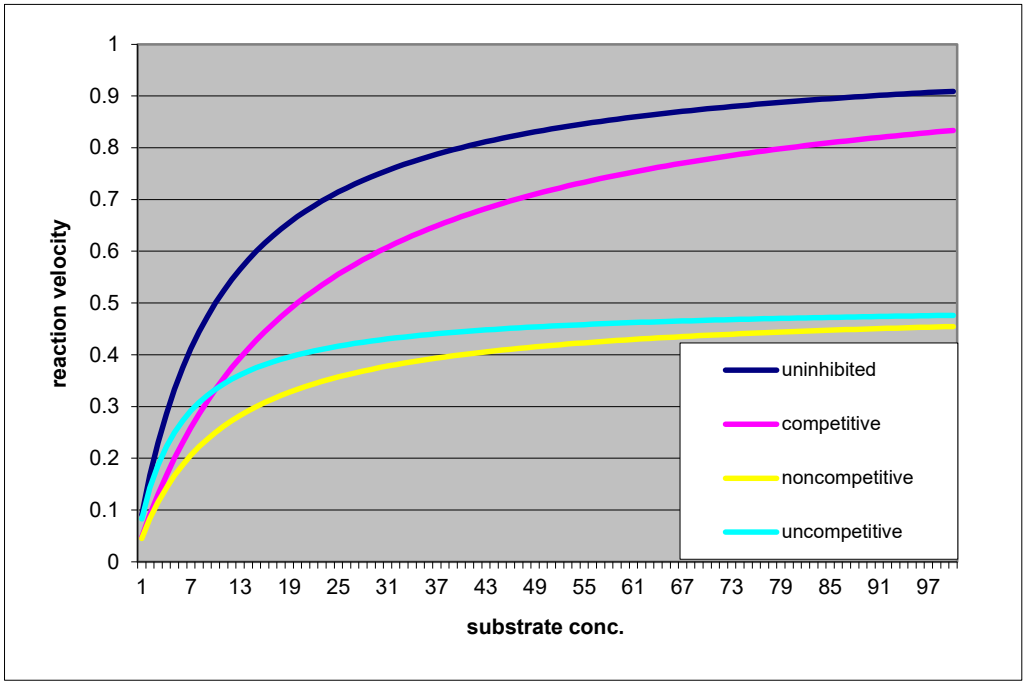
divide everything by $k_1$, then rearrange

$$x = \frac{s e_0}{\left(K_S + s\left(1 + i/K_i\right)\right)} = e_0 \left(\frac{1}{1 + i/K_i}\right) \frac{s}{\frac{K_S}{1 + i/K_i} + s};\ K_S = \frac{k_{-1}}{k_1}$$

And thus, the reaction rate $V = k_2 x =$

$$V = V_{max} \frac{1}{1 + [I]/K_i} \frac{[S]}{\frac{K_s}{1 + [I]/K_i} + [S]};\ K_i = k_{-3}/k_3 \text{ and } K_s = k_{-1}/k_1$$

## Comparing inhibitor behavior:




These were generated with $[I]/K_I = 1$, $K_s=10$. Things to look at: maximum velocity, which is approached for each situation, and will be either 1 or 0.5. Also substrate concentration at which velocity is half of the value approached in that situation. Compare against forms presented above, and justify.

## *Lineweaver-Burk plots*

Okay, given a set of reaction data, how do we get rate constants? Sounds simple to find the substrate concentration that gives maximum reaction velocity. However, that velocity is never reached.

Take the reciprocal of both sides:

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

becomes

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

From this Lineweaver-Burk plot, we can get $V_{max}$ and $K_M$ from the slope and intercept.

A similar approach can be used to measure $K_{eq}$ for the biomolecular binding systems discussed earlier.

However, note that measurements at small [S] and V are prone to large errors and have a large input on these curves. One can go through sensitivity analysis of this system, but it's not pretty. In my opinion, using contemporary curve fitting software provides much more reliable results.

This brings the fair point of why to study this traditional treatment at all. Why not use programs for solving differential equations? Here are two good reasons:

- The concept of $K_M$ is very useful for comparing and characterizing reactions
- The statements about the wide range of rate constants are true, and pose difficult challenges in numerical solutions; uniting timescales that vary over several orders of magnitude is tricky