Bio-Systems Modeling and Control

Lecture 23
Enzymes
Inhibition and Activation

Controlling Enzyme Activity

- The activity level of many enzymes can be enhanced or decreased by certain molecules, acting either as activators or inhibitors.
- The therapeutic effects of many drugs depend on their ability to inhibit specific enzymes, thereby blocking metabolic or physiological processes involved in a disease.

Example: Enzyme Inhibition associated with an Antidepressant Drug

- The enzyme monoamine oxidase (MAO) breaks down serotonin.
- High levels of serotonin in the brain are associated with “positive mood”.
- The antidepressant drug tranylcypromine (Parnate) inhibits the MAO enzyme.
- By inhibiting MAO, the level of serotonin in the brain increases, and mood improves.

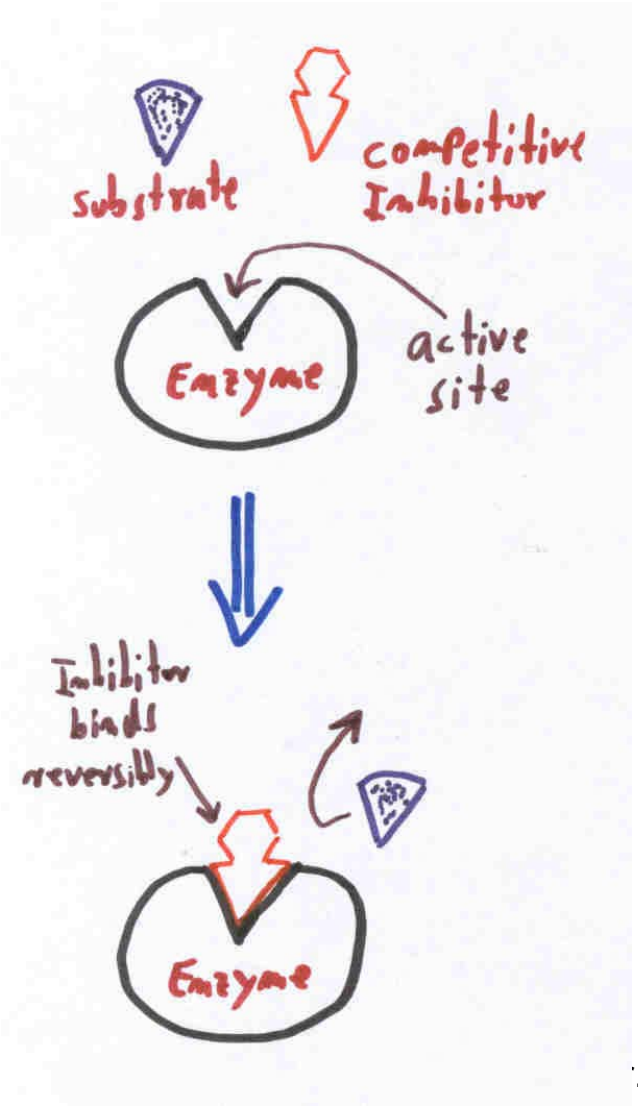
Irreversible Enzyme Competitive Inhibition

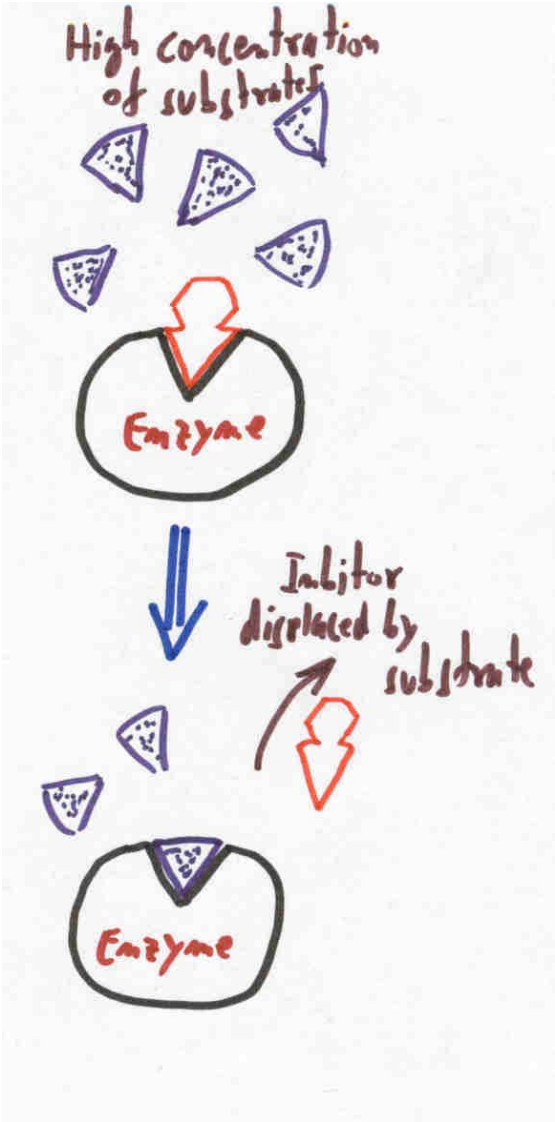
- Inhibitor molecules may form stable covalent bonds with groups inside the enzyme's active site, thereby blocking formation of E+S complexes.
- Some toxins (cyanide and many nerve gases) fall into this category.
- For normal cell function, we need reversible inhibition.

(Reversible) Competitive Inhibition

- It is caused by molecules that have similar shape as that of the substrate, which compete with the substrate molecules interacting with enzyme active site.
- Inhibition can be reversed, if substrate concentration is increased. This may even cause a displacement of the inhibiting molecules out of the enzyme's active site.

Competitive Inhibition Illustrated [based on Eckert's Animal Physiology]

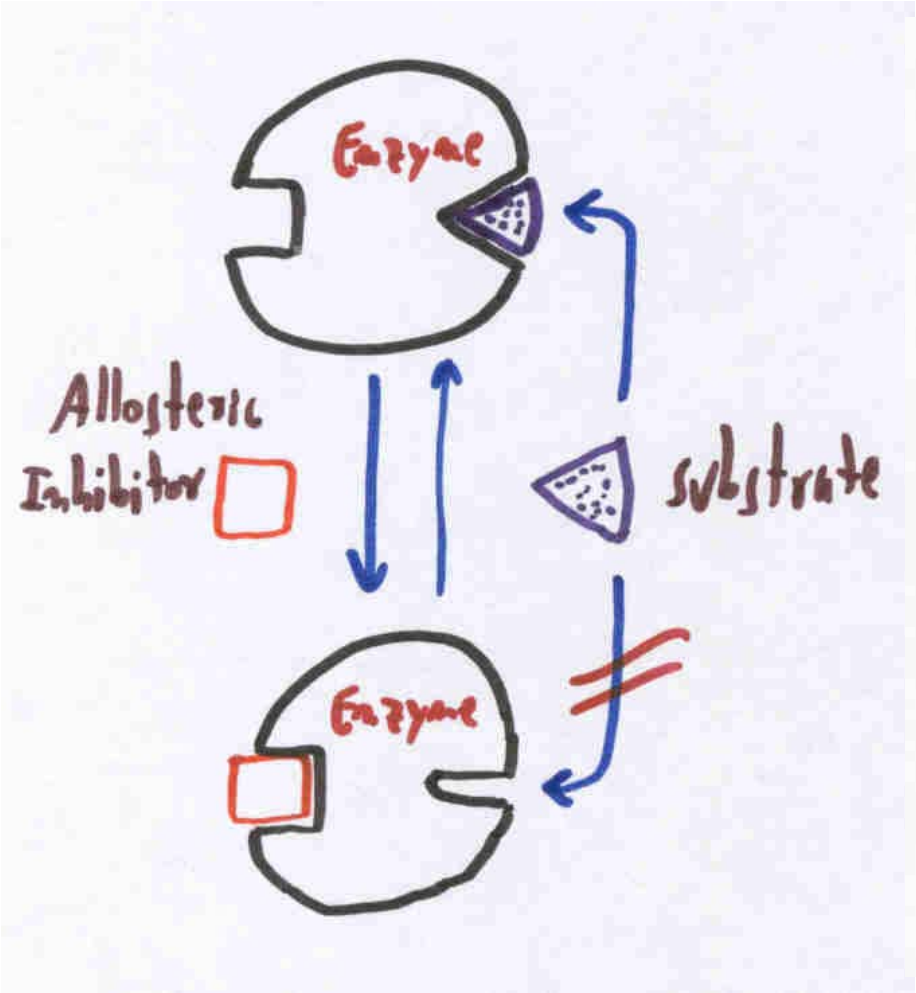


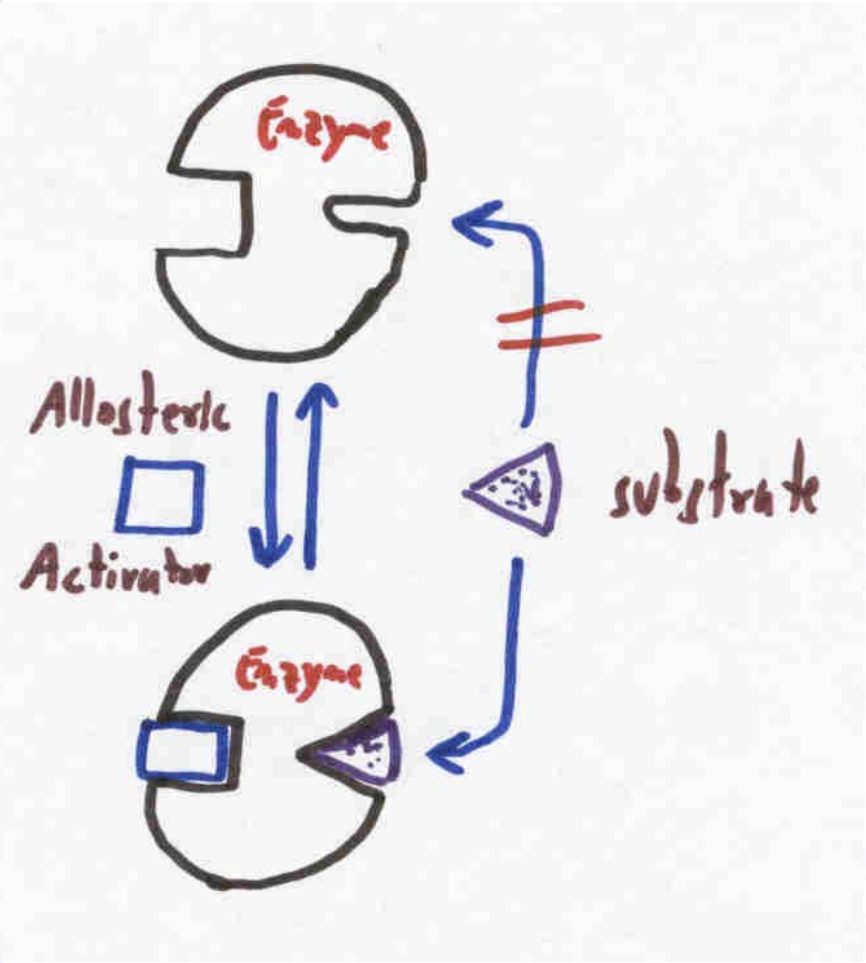


Noncompetitive Inhibition

- This type of enzyme inhibition is caused by molecules (other than the substrate) that bind to regions in the enzyme outside the substrate active site.
- More common name to such inhibition is allosteric inhibition ("allosteric" = from Greek: "another solid").
- Such inhibition effect cannot be reversed by changing substrate's concentration – only by dilution or removal of inhibitor.

Allosteric Inhibition and Activation Illustrated
[based on Eckert's Animal Physiology]



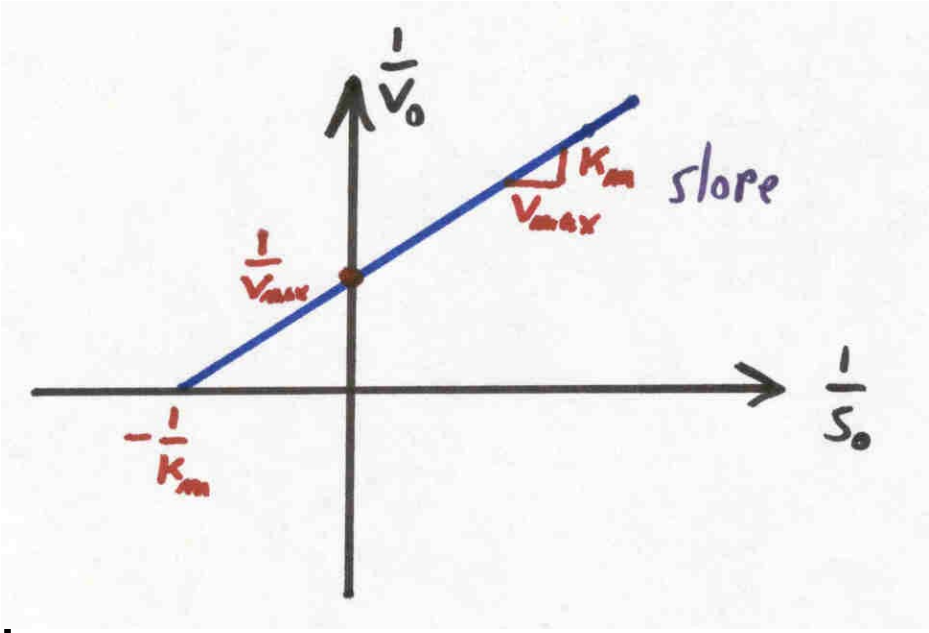


Allosteric Interactions Explained

- An activator or inhibitor molecule, as it combines with the enzyme, at its allosteric site, may cause configuration alteration of the enzyme's molecule.
- Allosteric Inhibitor causes the active site (that normally fits a substrate) to "shrink" (or lose its compatibility in other ways).
- Allosteric Activator enables the active site (which is normally disabled).

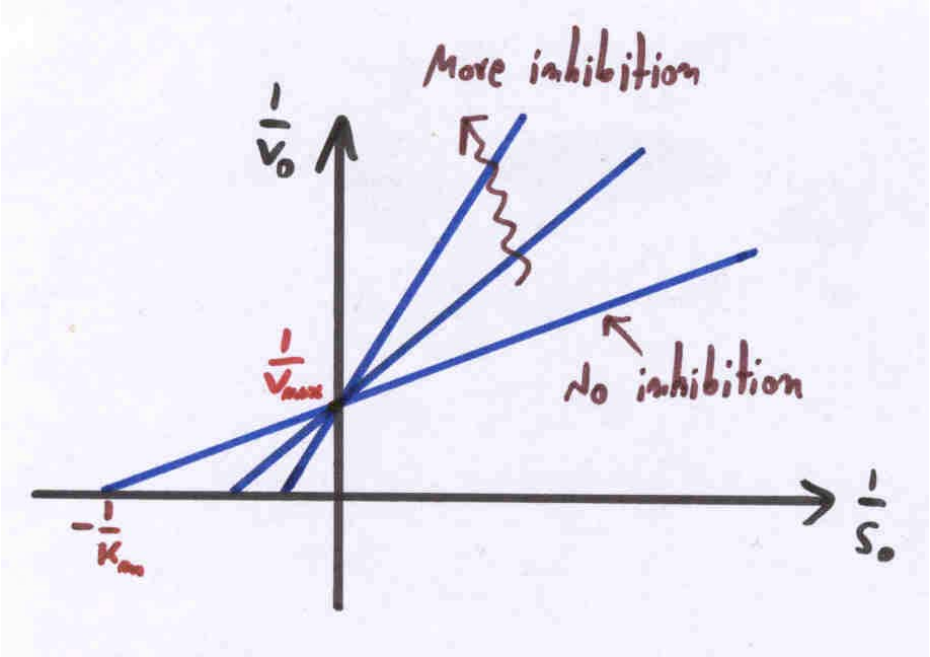
Recall: The Lineweaver-Burk Plot of Simple Enzyme Kinetics

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}} \cdot \frac{1}{s_0} + \frac{1}{V_{\max}}$$



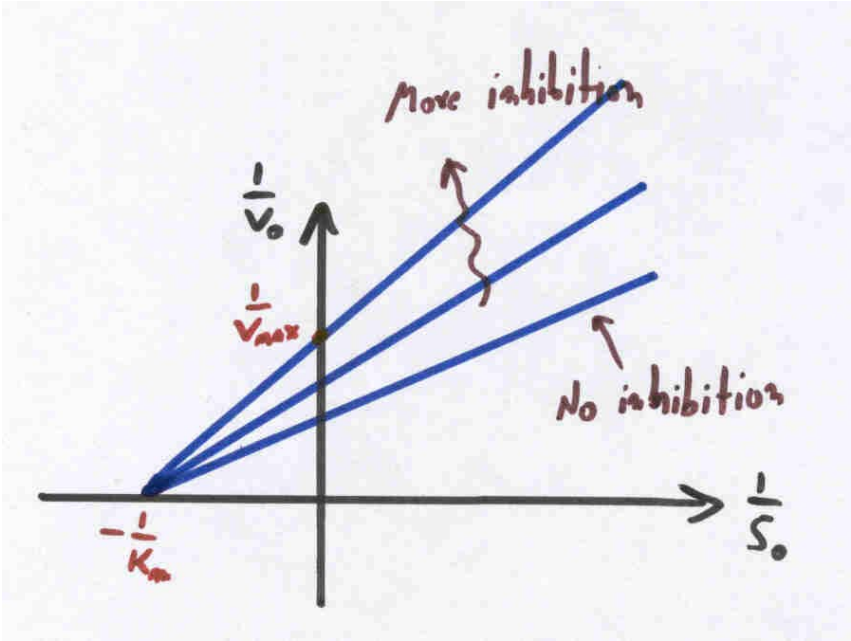
- K_m and V_{max} are the unknowns.
- Only two experiments (with two different values of s_0) are needed – we then find the unknowns from the x-axis and y-axis intercepts of the line.

Effect of Competitive Inhibition on the Lineweaver-Burk Plot: Slope=K_m/V_{max} increases (to be proved soon)



- Competitive inhibition increases Michaelis constant K_m, but does not change V_{max}.
- Only x-intercept is affected. Y-intercept unchanged.

Effect of Allosteric Inhibition on the Lineweaver-Burk Plot: Slope=K_m/V_{max} increases (to be proved soon)



- Allosteric inhibition decreases V_{max} but does not alter K_m. Reduction of V_{max} is equivalent to reduction of enzyme concentration e_0.
- Only y-intercept is affected.

Mathematical Details of Competitive and Allosteric Inhibition

Reaction Rate Equations, using Michaelis-Menten quasi-steady-state relationships

Competitive Inhibition of Enzyme Activity

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

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

- Inhibitor is I. Assume that $k_1'=k_1$ and $k_{-1}'=k_{-1}$. That is, reaction rates for E,S are not affected by I. Rates k_3 and especially k_{-3} may depend on s.
- C_1 is the substrate-enzyme complex, whereas C_2 is the inhibitor-enzyme complex.

Competitive Inhibition Reaction Rate Equations

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

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

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

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

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

$$\frac{de}{dt} = -(k_1' s + k_3 i) e + (k_{-1}' + k_2) c_1$$

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

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

Initial conditions:
$s(0)=s_0$,
$e(0)=e_0$,
$i(0)=i_0$
$c_1(0)=c_2(0)=$
$=p(0)=0$

Again $e+c_1+c_2=e_0$ and we can eliminate the e equation and look later at p equation

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

$$\frac{dc_2}{dt} = k_3ie - k_{-3}c_2$$

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

$$\frac{di}{dt} = -k_3ie + k_{-3}c_2$$

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

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

ICs: $s(0)=s_0$, $i(0)=i_0$, $c_1(0)=c_2(0)=0$

We already know, by Michaelis-Menten theory that c_1 and c_2 quickly reach quasi-steady-state. Therefore we apply $dc_1/dt \approx dc_2/dt \approx 0$ and get:

$$c_1 \approx \frac{K_i e_0 s}{K_m i + K_i s + K_m K_i}$$

$$c_2 \approx \frac{K_m e_0 i}{K_m i + K_i s + K_m K_i}$$

$$K_m = \frac{k_{-1}' + k_2}{k_1'} \qquad K_i = \frac{k_{-3}}{k_3}$$

We can now find the speed of reaction V, from $dp/dt = k_2 c_1$

Quasi-Steady-State for c_1,c_2: Reaction Velocity

$$c_1 \approx \frac{K_i e_0 s}{K_m i + K_i s + K_m K_i}$$

$$c_2 \approx \frac{K_m e_0 i}{K_m i + K_i s + K_m K_i}$$

$$K_m = \frac{k_{-1}' + k_2}{k_1'} \qquad K_i = \frac{k_{-3}}{k_3}$$

$$\boxed{V} = \frac{dp}{dt} = k_2 c_1 = \frac{k_2 e_0 s K_i}{K_m i + K_i s + K_m K_i} = \boxed{\frac{V_{\max} s}{s + K_m (1 + \frac{i}{K_i})}}$$

Competitive Inhibition Reaction Velocity Explained

$$V = \frac{V_{\max} s}{s + K_m (1 + \frac{i}{K_i})}$$

- We see that Michaelis-Menten constant is increased from K_m to $K_m(1+i/K_i)$.
- We also see that the maximum velocity V_{max} does not change.
- This explains the Lineweaver-Burk plots that we saw earlier. An increase in s may cause $K_i=k_{-3}/k_3$ to increase.

Bottom Line Simplified Model for Competitive Inhibition

$$\frac{dp}{dt} \approx \frac{V_{\max} s}{s + K_m (1 + \frac{i}{K_i})}$$

- Where s(t) and i(t) are the two process inputs
- Can i be replaced by some constant i_{eq} representing some steady-state value of i(t)?
- If $k_3 >> k_{-3}$ then $c_2(t)$ approaches the value i_0 very quickly and then i(t) → 0 quickly, which partially defeats the inhibition action.
- It is important that k_{-3} is not too small!

How to Model Allosteric Inhibition?

- Possible idea: Same rate equations, as for Competitive Inhibition, except that we now assume that the inhibitor is capable of altering the reaction rates k_1 and k_{-1}.
- Method chosen: Assume the existence of a transitory complex (EIS) and develop equations for rates of change of all complexes (ES,EI and EIS)

Plausible Model of Allosteric Inhibition of Enzyme Activity

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

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

$$S + C_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_3$$

$$C_1 + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_3$$

C_1 is the substrate-enzyme complex, C_2 is the inhibitor-enzyme complex and <u>C_3 is the inhibitor-substrate-enzyme complex</u>.

Allosteric Inhibition Reaction Rate

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

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

$$S + C_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_3$$

$$C_1 + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_3$$

Initial conditions:
$s(0)=s_0$, $e(0)=e_0$,
$i(0)=i_0$
$c_1(0)=c_2(0)=c_3(0)$
$=p(0)=0$

$$\frac{dc_1}{dt} = k_1 es + k_{-3}c_3 - (k_{-1} + k_2 + k_3 i)c_1$$

$$\frac{dc_2}{dt} = k_3 ie + k_{-1}c_3 - k_{-3}c_2$$

$$\frac{dc_3}{dt} = k_3 ic_1 + k_1 sc_2 - (k_{-1} + k_{-3})c_3$$

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

$$\frac{de}{dt} = -(k_1 s + k_3 i)e + (k_{-1} + k_2)c_1 + k_{-3}c_2$$

$$\frac{di}{dt} = -k_3 ie + k_{-3}(c_2 + c_3)$$

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

Total amount of enzyme: $e+c_1+c_2+c_3=e_0$
Define: $K_s=k_{-1}/k_1$ and $K_i=k_{-3}/k_3$

$$(e_0 - c_1 - c_2 - c_3)s - K_s c_1 \approx 0$$

$$(e_0 - c_1 - c_2 - c_3)i - K_i c_2 \approx 0$$

$$c_2 s - K_s c_3 \approx 0$$

$$c_1 i - K_i c_3 \approx 0$$

The above equations represent a strong assumption that complexes' concentrations quickly reach equilibrium [according to book by Keener and Sneyd]

Results of Quasi-Steady-State Analysis

$$c_1 \approx \frac{K_i e_0}{K_i + i} \cdot \frac{s}{K_s + s}$$

$$K_s = \frac{k_{-1}}{k_1} \qquad K_i = \frac{k_{-3}}{k_3}$$

We can now find the speed of reaction V, from $dp/dt = k_2 c_1$

Approximated Allosteric Inhibition Reaction Velocity

$$V = \frac{V_{\max}}{1 + \frac{i}{K_i}} \cdot \frac{s}{K_s + s}$$

- We see that Michaelis-Menten constant K_s remains unchanged. It is only an approximation!
- However the maximum velocity reduces from V_{max} to $V_{max}/(1+i/K_i)$.
- This explains the Lineweaver-Burk plots that we saw earlier.

Bottom Line Simplified Model for Allosteric Inhibition

$$\frac{dp}{dt} \approx \frac{V_{\max}}{1+\frac{i}{K_i}} \cdot \frac{s}{K_s + s}$$

- Again, can i be replaced by a constant value?
- Like in competitive inhibition it is important that the affinity of I to E is not too strong (that is k_3 should not be much larger than k_{-3})
- The larger k_{-3} the larger is i_{eq} approaching i_0.

Allosteric Activation

- With activation, the enzyme molecule can host both the substrate and the activator.
- Need to take into account different types of complexes, as in the above Allosteric Inhibition model.

Plausible Model of Allosteric Activation of Enzyme Activity

$$S + E \underset{k_{-1} \to 0}{\overset{k_1 = 0}{\rightleftharpoons}} C_1$$

$$E + A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2$$

$$S + C_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} C_3$$

$$C_3 \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} C_1 + A$$

$$C_1 \xrightarrow{k_2} E + P$$

C_1 = SE, C_2 = EA, C_3 = SEA . C_1 can come only out of C_3. We assume that $k_1 = 0$.

Simplified Model of Allosteric Activation of Enzyme Activity

$$E + A \xrightarrow{k_3} C_2$$

$$S + C_2 \xrightarrow{k_4} C_3$$

$$C_3 \xrightarrow{k_5} C_1 + A$$

$$C_1 \xrightarrow{k_2} E + P$$

For simplicity let us neglect the rates k_{-1}, k_{-3}, k_{-4}, k_{-5} . One can later repeat the derivation without such an assumption.

Allosteric Activation Reaction Rate

$$E + A \xrightarrow{k_3} C_2$$

$$S + C_2 \xrightarrow{k_4} C_3$$

$$C_3 \xrightarrow{k_5} C_1 + A$$

$$C_1 \xrightarrow{k_2} E + P$$

Initial conditions:
$s(0)=s_0$, $e(0)=e_0$,
$a(0)=a_0$
$c_1(0)=c_2(0)=c_3(0)$
$=p(0)=0$

$$\frac{dc_1}{dt} = k_5 c_3 - k_2 c_1$$

$$\frac{dc_2}{dt} = k_3 ae - k_4 c_2 s$$

$$\frac{dc_3}{dt} = k_4 s c_2 - k_5 c_3$$

$$\frac{ds}{dt} = -k_4 c_2 s$$

$$\frac{de}{dt} = -k_3 ae + k_2 c_1$$

$$\frac{da}{dt} = -k_3 ae + k_5 c_3$$

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

Allosteric Activation Constraint

$$E + A \xrightarrow{k_3} C_2$$

$$S + C_2 \xrightarrow{k_4} C_3$$

$$C_3 \xrightarrow{k_5} C_1 + A$$

$$C_1 \xrightarrow{k_2} E + P$$

$d/dt(e+c_1+c_2+c_3)=0 \rightarrow$

$\rightarrow$

$e(t)+c_1(t)+c_2(t)+c_3(t)=e_0$

$$\frac{dc_1}{dt} = k_5 c_3 - k_2 c_1$$

$$\frac{dc_2}{dt} = k_3 ae - k_4 c_2 s$$

$$\frac{dc_3}{dt} = k_4 s c_2 - k_5 c_3$$

$$\frac{ds}{dt} = -k_4 c_2 s$$

$$\frac{de}{dt} = -k_3 ae + k_2 c_1$$

$$\frac{da}{dt} = -k_3 ae + k_5 c_3$$

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

Michaelis-Menten Quasi-Steady-State Relationships for Allosteric Activation

$$\frac{dc_1}{dt} \approx 0 \rightarrow c_3 \approx \frac{k_2}{k_5} c_1$$

$$\frac{dc_3}{dt} \approx 0 \rightarrow c_2 \approx \frac{k_2 c_1}{k_4 s}$$

$$\frac{dc_2}{dt} \approx 0 \rightarrow c_1 \approx \frac{k_3 k_5 e_0 as}{k_3(k_5 + k_2)as + k_2 k_3 a + k_4 k_2 s}$$

$$\frac{dp}{dt} = k_2 c_1 \approx \frac{k_2 k_3 k_5 e_0 as}{k_3(k_5 + k_2)as + k_2 k_3 a + k_4 k_2 s}$$

Allosteric Activation: Extreme Case 1

$$Let: _ k_3 a >> k_4 s$$

$$\frac{dp}{dt} \approx \frac{k_2 k_3 k_5 e_0 as}{k_3(k_5 + k_2)as + k_2 k_3 a + k_4 k_2 s} = \frac{k_2 k_5 e_0 s}{(k_5 + k_2)s + k_2}$$

$$V_{\max,old} = k_2 e_0 \qquad K_{m,old} = \frac{k_2}{k_1}$$

$$V_{\max,new} = \frac{k_2 k_5 e_0}{k_5 + k_2} \qquad K_{m,new} = \frac{k_2}{k_5 + k_2}$$

Allosteric Activation: Extreme Case 2

$$Let: _ k_3 a << k_4 s$$

$$\frac{dp}{dt} \approx \frac{k_2 k_3 k_5 e_0 as}{k_3(k_5 + k_2)as + k_2 k_3 a + k_4 k_2 s} = \frac{k_2 k_3 k_5 e_0 a}{k_3(k_5 + k_2)a + k_4 k_2}$$

Allosteric Activation Simplified Model

$$\frac{dp}{dt} \approx \frac{k_2 k_3 k_5 e_0 a s}{k_3(k_5 + k_2)as + k_2 k_3 a + k_4 k_2 s} = \frac{V_{\max,new} s}{s + K_{m,new}}$$

$$V_{\max,old} = k_2 e_0 \qquad K_{m,old} = \frac{k_2}{k_1}$$

$$V_{\max,new} = \frac{k_2 k_3 k_5 e_0 a}{k_3(k_5 + k_2)a + k_4 k_2} \qquad K_{m,new} = \frac{k_2 k_3 a}{k_3(k_5 + k_2)a + k_4 k_2}$$

It seems that the main influence of A is to make K_m smaller, however V_{max} gets slightly smaller as well.

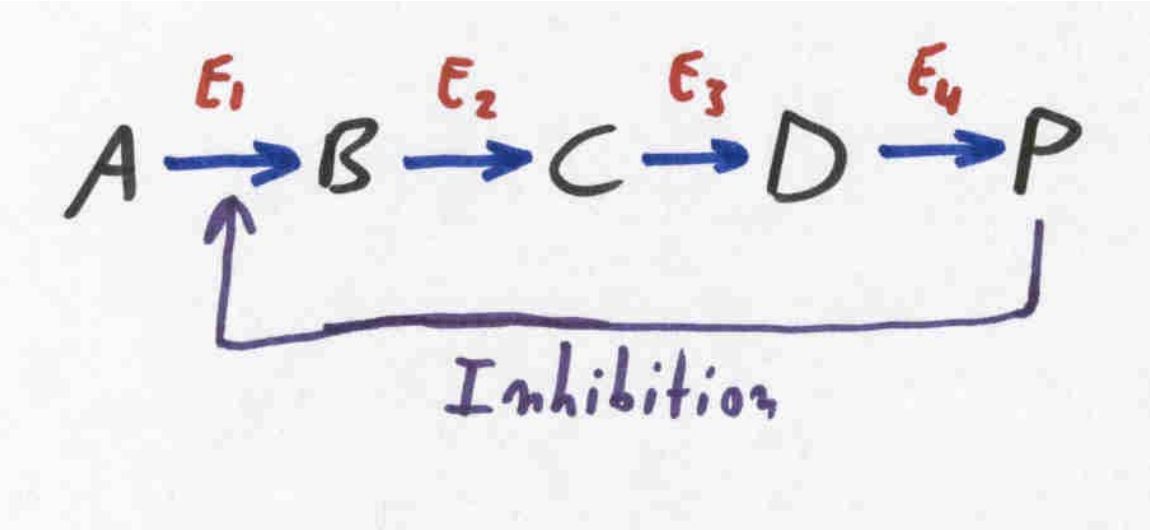
Comment about the activation model

- As in the discussion of inhibition models, it is important that a(t) does not go to zero.
- In the previous derivation we neglected both k_{-3} and k_{-5} which works against the activation action.
- One of the above reverse reaction rates must be sizeable enough.
- Need to rederive the model accordingly.

What restricts accumulation of the Product?

- In all the models that we have seen thus far, the accumulation of product P has no effect on the reaction rate.
- Need to regulate reaction rate by accumulation of end product.
- In many metabolic pathways, <u>end product P acts as inhibitor</u> to one of the reaction's enzymes.

End Product Feedback Inhibition



- Many reactions are multi-step enzymatic reactions.
- Often, the first enzyme (E_1 above) acts as a regulatory enzyme. P often acts as allosteric inhibitor of E_1.