

Biomeng 261. Lecture 3.

Enzyme regulation: inhibition & activation
(mainly)

'We' (ie our bodies, nature etc)
don't just want enzymes
to be 'always on'

→ they are tightly
controlled

↳ adapt to needs &
resources

How?

- Amount

↳ eg via gene expression
(see later)

- Activity

↳ Regulatory molecules:

activator: turn 'up'

inhibitor: turn 'down'

Regulation of activity: types

- irreversible (eg toxins)

↳ not usually for control

↳ typically covalent (strong)

main focus

- reversible

↳ good for control

↳ typically non-covalent (weaker)

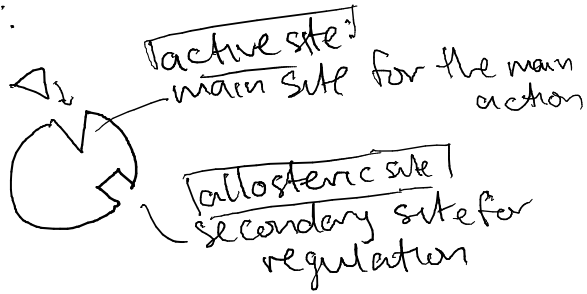
Reversible regulation: more terminology:

behaviour {
- competitive ≠ today
- non-competitive
- un-competitive
- mixed

location {
- active site
- allosteric site

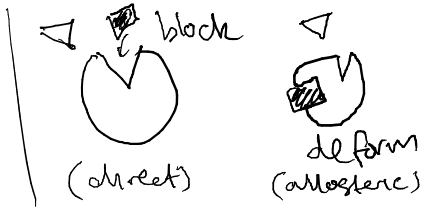
Locations:

allos:
'other'
sterEOS:
'solid object'



Behaviour: competitive vs un vs non

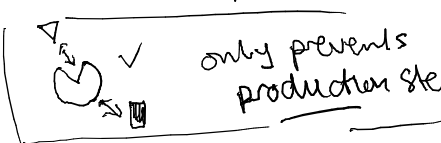
- competitive:
 - Substrate & inhibitor can't bind enzyme at same time
 - Can be 'direct' or allosteric competition



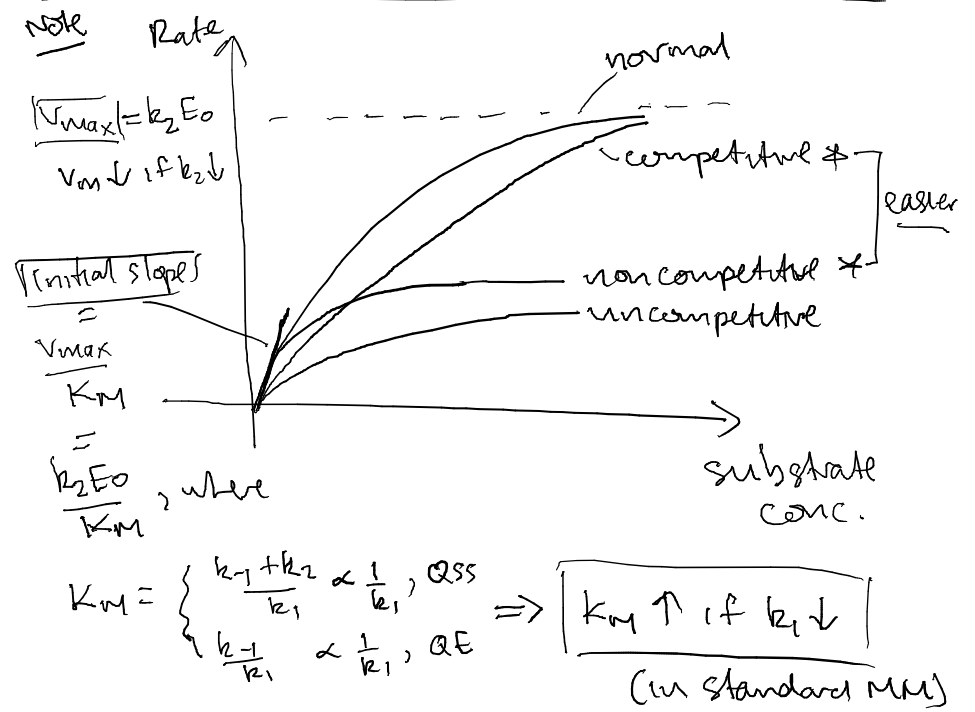
- uncompetitive:
 - Binds only to complex
 - slows unbinding & production reactions
 - Reduces available 'active' enzymes & substrate



- non competitive:
 - Binds to either (both) enzyme & complex
 - only slows production step.



Will show get same final form but modified constants i.e



Goal: derive effects for simple models of inhibition:

- competitive (V_{max} same, $K_M \uparrow$)
- non-competitive ($V_{max} \downarrow$, K_M same)

→ will be like changing MM
→ but based on more complex models

Competitive inhibition model

Same idea as building MM model

Simple but larger systems

↓

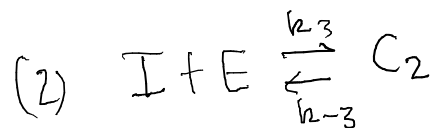
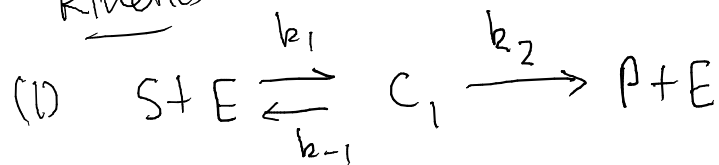
reduce to smaller but
modified system

Assume

- Inhibitor I directly competes with substrate S .

⇒ Two types of complex $\begin{cases} C_1 = E:S \\ C_2 = E:I \end{cases}$

Kinetics:



Competitive inhibition model cont'd

1. Conservation $[S], [I], [C_1], [C_2], [E], [P]$

$$\frac{d[S]}{dt} = -J_1 + J_{-1}$$

$$\frac{d[I]}{dt} = -J_3 + J_{-3}$$

$$\frac{d[C_1]}{dt} = +J_1 - J_{-1} - J_2$$

$$\frac{d[C_2]}{dt} = +J_3 - J_{-3}$$

| | |
|-------------------------------------|--|
| can always eliminate $[E]$ | $\frac{d[E]}{dt} = (-J_1 + J_{-1} + J_2) + (-J_3 + J_{-3})$ $= -\frac{d[C_1]}{dt} - \frac{d[C_2]}{dt}$ $\Rightarrow [E] + [C_1] + [C_2] = E_0$ |
|-------------------------------------|--|

$$v := \frac{d[P]}{dt} = +J_2 \quad \boxed{\text{goal}}$$

2. Using mass action

[exercise!]

gives

$$\bullet \frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[C_1]$$

$$\bullet \frac{d[I]}{dt} = -k_3[E][I] + k_{-3}[C_2]$$

$$\bullet \frac{d[C_1]}{dt} = k_1[E][S] - (k_{-1} + k_2)[C_1]$$

$$\bullet \frac{d[C_2]}{dt} = k_3[E][I] - k_{-3}[C_2]$$

$$\& \bullet [E] = E_0 - [C_1] - [C_2]$$

$$\bullet v = \frac{d[P]}{dt} = k_2[C_1] \quad (\text{uncomplexed})$$

Note:

(use $E = f(C_1, C_2)$)

$$1. \frac{d[S]}{dt} = f_1(\underline{C_1, C_2}, S)$$

$$2. \frac{d[I]}{dt} = f_2(\underline{C_1, C_2}, I)$$

$$3. \frac{d[C_1]}{dt} = f_3(\underline{C_1, C_2}, S)$$

$$4. \frac{d[C_2]}{dt} = f_4(\underline{C_1, C_2}, I)$$

$$5. \frac{d[P]}{dt} = f_5(\underline{C_1})$$

Goal: eliminate enzyme complex
too (C_1 & C_2)

\Rightarrow need two conditions/approx.

$$\left| \begin{array}{l} \bullet QSS \ C_1 \\ \bullet QSS \ C_2 \end{array} \right|$$

① $[C_1]$ QSS ie $\boxed{\text{set } \frac{d[C_1]}{dt} \approx 0 \text{ relative to } \frac{d[S]}{dt} \text{ (see also later)}}$

$$\Rightarrow k_1 [E][S] - (k_{-1} + k_2)[C_1] \approx 0$$

$$\Rightarrow \boxed{k_1 (E_0 - [C_1] - [C_2])[S] - (k_{-1} + k_2)[C_1] = 0}$$

② $[C_2]$ QSS

$$\Rightarrow \boxed{k_3 (E_0 - [C_1] - [C_2])[I] - k_{-3}[C_2] = 0}$$

two equations \Rightarrow eliminate two variables C_1, C_2 .

$\left. \begin{array}{l} \vdots \\ \text{details!} \\ \text{(exercise!)} \\ \vdots \end{array} \right\} \text{tutorial Q} \quad \text{😊}$

\Rightarrow

$$[C_1] = \frac{K_I E_0 [S]}{K_M [I] + K_I [S] + K_M K_I}$$

$$[C_2] = \frac{K_M E_0 [I]}{K_M [I] + K_I [S] + K_M K_I}$$

where $\boxed{K_I = \frac{k_{-1}}{k_1}}$
 $K_M = \frac{k_{-1} + k_2}{k_1}$

so

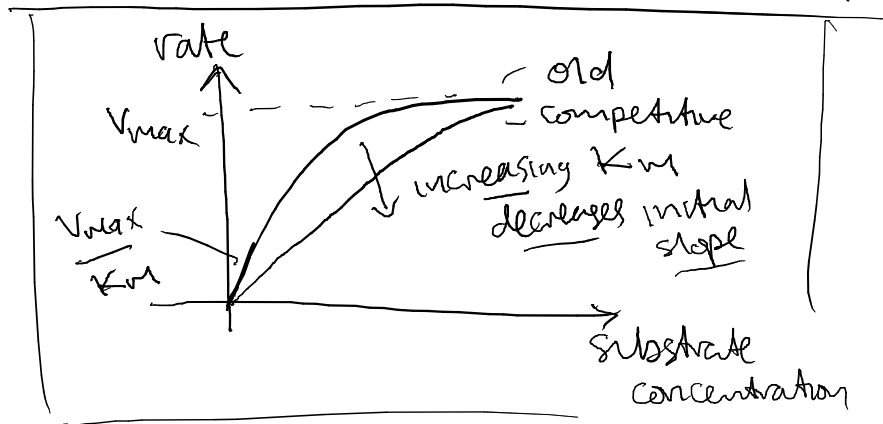
$$\begin{aligned} \sim \frac{d[P]}{dt} &= k_2 [C_1] \\ &= \frac{k_2 K_I E_0 [S]}{K_M [I] + K_I [S] + K_M K_I} \\ &= \frac{K_I (k_2 E_0 [S])}{K_I (K_M (1 + \frac{[I]}{K_I}) + [S])} \end{aligned}$$

$$\Rightarrow v = \frac{k_0 E_0 [S]}{K_M (1 + \frac{[I]}{K_I}) + [S]}$$

ie

$$v = \frac{V_{max} [S]}{K_M^{new} + [S]}$$

$$\Rightarrow \text{Same } V_{max} \text{ new } K_M \text{ (} K_M^{new} > K_M^{old} \text{)}$$



Next time: noncompetitive inhibition
(& other complications)

A note on the quasi-steady-state approximation.

what's the difference between a

$$\begin{array}{l} \text{'quasi' steady state } \frac{dc}{dt} \approx 0 \\ \& \text{ a} \\ \text{'true' steady state } \frac{dc}{dt} = 0 \end{array}$$

• Easy case: true steady state

$$\left[\frac{dc}{dt} = 0 \right] \text{ exactly zero.}$$

• Subtle case: 'quasi'

→ 'approximately' zero means
small relative to
something else

→ what is the something else?

Relative smallness

- we are using the QSS to approximately eliminate a variable relative to others

eg

$$\left| \frac{\frac{dc}{dt}}{\frac{ds}{dt}} = \text{small} \right|$$

- BUT the relative change on its 'own' scale might still be significant!

$$\frac{dc}{dt} / \frac{\Delta c_{\max}}{T} \approx 1$$

\Rightarrow Key is Δc_{\max} small relative to Δs_{\max}

'Dynamic' steady state

$\frac{dc}{dt}$: changes fast on its own scale

- rapidly reaches steady state on its own scale

- these changes are small ($\sim \epsilon_0$) in magnitude relative to long term changes in other variables
- if the other scale is changing, forces c to readjust

$\frac{ds}{dt}$: changes on its own, slower scale

- these changes are larger ($\sim s_0$)
- force c to constantly readjust - 'dynamic steady state'

\rightarrow c's steady state is 'slaved' to s

& c can be eliminated using

$$c_{ss} = f(s) \quad \text{via} \quad \frac{dc}{dt} \approx 0$$

subject of 'singular perturbation theory' EngSci711

QSS summary: 3 key / typical features

- a variable can be set to quasi-steady state when it rapidly reaches its own steady-state value relative to other variables of primary interest ①
- usually the magnitude of its changes in reaching SS are small relative to the changes of other variables in reaching their SS. ②
- changes in other variable cause readjustment: 'dynamic steady state' ③

eg • $\frac{dc}{dt} / \frac{dc}{dt} \big|_{\max} \approx 1$

BUT

• $\frac{dc}{dt} / \frac{ds}{dt} \ll 1$

- as $C \rightarrow P$ 'slowly', $S+E \rightarrow C$ adjusts 'quickly' to keep balance ie more complex is made

Here:

$$\frac{E_0}{S_0} \ll 1$$

&

$$\frac{dc}{dt} \approx \frac{E_0}{T}$$

How to non-dimensionalise

$$C = C_0 C^* = \text{scale} \times \text{non-dim var}$$

$$t = T t^*$$

$$\frac{dC}{dt} = \frac{d(C_0 C^*)}{d(T t^*)} = \frac{C_0}{T} \frac{dC^*}{dt^*}$$

similarly

$$\frac{dS}{dt} = \frac{S_0}{T} \frac{dS^*}{dt^*}$$

- always a valid non-dimensionalisation


- but, called 'well-scaled'

if [also]

$$\left[\begin{array}{l} \frac{dC^*}{dt^*} \approx 1, \text{ i.e. } \frac{dC}{dt} \sim \frac{C_0}{T} \\ \frac{dS^*}{dt^*} \approx 1, \text{ i.e. } \frac{dS}{dt} \sim \frac{S_0}{T} \end{array} \right]$$

⇒ determines 'regime' where scaling is 'good'

QSS:

use $C_0 = E_0$ 

C rapidly reaches its max E_0

$$\frac{dC}{dt} / \frac{dS}{dt} \approx \frac{E_0}{S_0}$$

'good' if $E_0/S_0 \ll 1$
⇒ C's max is small rel. to S's