Complex Reactions/Inhibition – 2014 – Notes 6

e.g. two enzyme substrate complexes (reactant & product)

\[
\begin{align*}
\text{fumarate (F)} & : \text{C} = \text{C} \quad \text{COO}^\cdot + \text{H}_2\text{O} \xrightarrow{\text{fumarase}} \text{malate (M)} : \text{C} - \text{C} \quad \text{COO}^\cdot \\
E + F & \xrightarrow{k_1} E F \quad E F \xrightarrow{k_2} E M \quad E M \xrightarrow{k_3} E + M
\end{align*}
\]

Analysis gives initial rate (messy to work out, mixes rapid equilibrium & steady state, do forward reaction only, let k_3 \approx 0):

\[
\nu_F = \frac{[k_2k_3/(k_2+k_2+k_3)]E_0][F]}{[k_1k_2+k_2k_3+k_2k_3]/[k_1(k_2+k_2+k_3)] + [F]} = \frac{V_F[F]}{K_M^F + [F]}
\]

result appears simple, same as Michaelis Menton form:

\[
\nu_{\text{MAX}}[S]/(K_M + [S]) \rightarrow \nu_{\text{MAX}} = V_F, \text{ but constants complex usual initial rate, experiment not separate intermediate}
\]

Aside: if do Relaxation measurement sense reverse rxn – analysis \rightarrow just do backwards, let "V_R" be max. rev. rate

\[
\nu_R = V_R[M]/(K_M^R + [M]) \quad \text{from} \quad \nu_R = d[F]/dt = k_1[EF]
\]

net rate (F-R) Remember? - did this considering Product, M

\[
\]

at beginning \rightarrow M = 0 -- gives back original form

at equilibrium:  
\[
V_FK_M^F[F]_e = V_RK_M^R[M]_e \quad K_e = [M]_e/[F]_e = V_FK_M^R / V_RK_M^F
\]

To parse out all k_1, k_2 ... etc. is difficult since observations combine Need to isolate/detect intermediates or vary conditions to get individual rate constants (ex. H/D exchange)
Inhibition
Sometimes path involves multiple intermediates
but still apparently simple (like above)
\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_2} ES' \xrightarrow{k_3} ES'' \xrightarrow{k_4} E + P
\]
\[v_0 = k_{\text{cat}}[E_0][S]/(K_M + [S])\]
Difference is \(k_{\text{cat}}\) and \(K_M\) combine all \(k_i\) above
If one step (i) is slow (rate limit) \(k_{\text{cat}} = k_i\)
\(\rightarrow\) turnover depends on slow step
\(K_M\) always like (apparent) dissociation constant
for all enzyme bound species: \([E][S]/[ES]\)
if \(k_1 \gg k_i\) then \(K_M = k_{-1}/k_1\) true dissociation const.

Now many enzymes bind several different substrates
but only one gives product
\(\rightarrow\) Some substrates called inhibitor, I, bind tightly
but have no reaction \(\rightarrow\) remove \(E_0\) - not avail. for S

Competitive inhibition, S and I bind to the same site
\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P
\]
\[
E + I \xrightarrow{k_3} EI
\]
This agrees with books and notes, reduces amount of \(E_0\)
available to create ES, depend on \(K_i\) –dissoc. const. of EI
**Competitive inhibition**, parallel mechanism

\[
E + S \xrightleftharpoons[k_1]{k^{-1}_1} ES \xrightarrow[k_2]{k^{-2}_2} E + P
\]

\[
E + I \xrightarrow[k_3]{k^{-3}_3} EI
\]

- Inhibitor (---) comparison to No Inhibitor (----) --
  - Competitive inhibition
    - plot rate \( v_0 \) vs. [S] and \( 1/v_0 \) vs. 1/[S] see comparison.
    - Inhibitor raises slope of L- B plot (dec. rate, \( 1/v_0 \) less) but same y intercept \( \rightarrow 1/v_m \) – change \( K_m \) not \( v_m \)
    - half velocity, \( v_0 = v_m/2 \), intercept on \( v_0 \) vs. [S] plot = \( K_m \)

*Two paths for \( E_0 \), result: less available \( E \) for making \( P \)*
do steady state on [ES] again -- still product only from $k_2$ step

$v = k_2[ES]$  \[ ES = \frac{k_1[E][S]}{(k_1 + k_2)} = K_M^{-1}[E][S] \] --as M-M

but now  \[ [E_0] = [E] + [ES] + [EI] \] reduce effect. enzyme, i.e $[E]$

(El ties up some of $E_0$ - prevents formation ES, lowers E)

let $K_i = [E][I]/[EI]$  \[ M = [E][S]/[ES] \] --2 dissoc const./paths

$[E_0] = [E](1 + [I]/K_i) + [ES]$  -- subst [EI] in $[E_0]$ eq.

not substitute for ES because will solve for it

[I] reduces $[E] + [ES]$ avail. -- consider $E \xrightarrow{\frac{k_1}{k_{1}} \text{ES}}$ ES equil.

Parameter for *Inhibitor* effect:  \[ \alpha = (1+[I]/K_i) = (1 + [EI]/[E]) \]

-- Note $\alpha > 1$, inc. [I] inc. $\alpha$,

\[ [E] = ([E_0]–[ES])/(1+[I]/K_i) = ([E_0] - [ES])/\alpha = \frac{[E]}{\alpha} \]

sub $[E]$ into $[ES]$:

\[ [ES] = K_M^{-1}[E][S] = K_M^{-1}[S][([E_0]–[ES])]/\alpha \]

rearrange - $[ES](\alpha K_M+[S])=[E_0][S] \Rightarrow [ES]=[E_0][S]/(\alpha K_M+[S])$

rate like before: \[ v_0 = k_2[E_0][S]/(\alpha K_M + [S]) = \frac{v_{\text{MAX}}}{(\alpha K_M/[S] + 1)} \]

now inc. $\alpha$, decrease $v_0$, but $\alpha K_M = K'_M$ means

1/$v$ vs. 1/S has *diff. slope* $(\alpha K_M/v_{\text{MAX}})$ but *same intercept*

i.e. change $K_m$ not $v_m$ - L-B plot intersect on y-axis at 1/$v_m$

- high $K_i$, *dissoc EI*, $\alpha = (1+[I]/K_i) \sim 1$, lots E, get same $v_m$

\[ 1/v = (K'_M / v_{\text{MAX}})(1/[S]) + 1/v_{\text{MAX}} \]

fit half rate $\Rightarrow K'_M$

As [I] increases \[ I \ll K_i \Rightarrow \alpha \sim 1 \] → ordinary M - M

\[ I \gg K_i \Rightarrow \alpha \sim [EI]/[E] \gg 1, \text{ so } [EI] \gg [E] \]
Three forms of inhibition are discussed in various texts:

**Competitive**

\[
\begin{align*}
E+S & \xrightarrow{k_1} ES \xrightarrow{k_2} E+P \\
E+I & \xrightarrow{k_3} EI
\end{align*}
\]

**non-competitive**

\[
\begin{align*}
E+S & \xrightarrow{k_1} ES \xrightarrow{k_2} E+P \\
E+I & \xrightarrow{k_3} EI
\end{align*}
\]

**uncompetitive**

\[
\begin{align*}
E+S & \xrightarrow{k_1} ES \xrightarrow{k_2} E+P \\
E+I & \xrightarrow{k_3} EI
\end{align*}
\]

as described above

here both EI and ESI
(if \(\alpha = \alpha'\), simple case)

here no EI form \((\alpha = 1)\)
but ESI form \((\alpha' > 1)\)

Change with inhibitor (——) compared to normal enzyme (——)

The alternative to competitive are **non-competitive** and **uncompetitive**. The difference is how an inhibitor interacts with the enzyme-substrate complex, and prevents product forming

**Non-competitive:** (derive Engel - pp 712-13)

\[
\begin{align*}
E + S & \xrightarrow{k_1} ES \xrightarrow{k_2} E + P \\
E + I & \xrightarrow{k_3} EI \\
ES + I & \xrightarrow{-} ESI
\end{align*}
\]
\[ v_0 = k_2[E_0][S]/[[S](1+[I]/K_{IS})+K_m(1+[I]/K_I)] \]
\[ = v_{\text{MAX}} [S]/( \alpha'[S] + \alpha K_M ) \]
where \( \alpha' = (1+[I]/K_{IS}) \), \( \alpha = (1+[I]/K_I) \), \( K_{IS} = [ES][I]/[EIS] \)

Again: \([I]=0 \rightarrow \alpha'=1 \) (MM), as \([I]\) inc. reaction slows, less P

In Lineweaver Burk, both slope and intercept change

\[ 1/v = (\alpha K_M/ v_{\text{MAX}})(1/[S]) + \alpha'/v_{\text{MAX}} \]

Here - if \( \alpha, \alpha'>1 \) are independent, \( \alpha \neq \alpha' \) i.e. \( K_I \neq K_{IS} \) then measured \( K_M \) will be different with inhibitor

*thus they do not have to intercept at x-axis at 1/ \( K_M \)

However, most books show intercept on x-axis (called -1/\( K_M \) )

between inhibited and no inhibitor for this, or \( \alpha = \alpha' \). Why?

*Tinoco* makes comment that they describe “simplest case” – true

*Engel* just asserts they intercept and \( K_M \) is not changed

*Atkins* points out this is a special case, i.e. \( \alpha, \alpha'>1 \) but, \( \alpha = \alpha' \)

Why do this? if \( I \) binds to a site away from the S binding site,

then it is likely that S does not affect the binding constant of \( I \)

if true then, \( K_I = K_{IS} \) and thus \( \alpha = \alpha' \rightarrow \) makes x-intercept be \( K_M \)

**Uncompetitive:** drop \[ E + I \xrightarrow{k_3} EI \] \( \rightarrow \alpha = 1, \alpha' > 1, \)

So in this case the inhibitor cannot bind enzyme alone, needs the substrate first. Without this step \( K_{M} = K_{M}' \) (\( \alpha = 1 \))

slope not changed, but intercept change \( (\alpha'/v_{\text{MAX}}) \)

Same equation, but Lineweaver-Burk plots are *parallel*

As Tinoco notes, this is a special case
Plot for non-competitive inhibition

(iff $\alpha = \alpha' > 1$)

$K_M$ same, x-intercept, or $v_0 = \frac{1}{2} v_{\text{MAX}}$ but slope changes

$v_{\text{max}}$ reduced – non-competitive inhibition

See shape change: two slopes, two y-intercept
Inhibition hugely important –
most biochemistry processes need enzymes →
– need specific reaction to go under control
– need operate with low concentration enzyme
– need fast response to S perturbation - on/off
control – bio feedback/signaling – gone wrong – “sick”
Drugs/pharma intercede → inhibit

elementary dihydrofolate reductase (See Engel pp. 713-14)

DHFR reduces 7,8 dihydrofolate (DHF) to 5,6,7,8-
tetrahydrofolate, a step in the biosynthesis of thymidine.
It is inhibited by methotrexate (MTX) – see structures:

Shape of MTX looks like DHF, but amine function is methylated,
prevents use of N-H in reduction mechanism
Used in cancer therapies, less thymidine, less cell division
With MTX need more substrate to get to half \( v_{\text{MAX}} \), or \( K_M' = \alpha K_M > K_M \) -- slows reaction, steeper slope \( 1/v \)

L-B: \[
1/v = (\alpha K_M/ v_{\text{MAX}})(1/[S]) + \alpha'/v_{\text{MAX}}
\]

**Problem:** Use the data below to determine the inhibition mechanism for DHFR and the maximum rate. If \( K_M \) for DHFR is 0.10 mM, what is \( K_I \)?

adding more inhibitor, see slower rate:

<table>
<thead>
<tr>
<th>[DHF] (mM)</th>
<th>Rate w/50 nM MTX (mM s(^{-1}))</th>
<th>Rate w/100 nM MTX (mM s(^{-1}))</th>
<th>Rate w/200 nM MTX (mM s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.38</td>
<td>5.56</td>
<td>3.72</td>
</tr>
<tr>
<td>6</td>
<td>8.84</td>
<td>7.38</td>
<td>5.55</td>
</tr>
<tr>
<td>9</td>
<td>9.46</td>
<td>8.29</td>
<td>6.65</td>
</tr>
<tr>
<td>12</td>
<td>9.79</td>
<td>8.84</td>
<td>7.38</td>
</tr>
</tbody>
</table>

MTX competitive inhibitor, slope change, same intercept

fit data at left:

Slope= 0.535 s
Intercept =0.091 mM\(^{-1}\)

\( v_{\text{MAX}} = 11 \) mM s\(^{-1}\)

\( K_M' = 5.89 \) mM

\( K_I = 3.4 \) pM

**MTX – binds strongly!**

--remember \( K_I \) and \( K_M \)

dissociation const, smaller value, stronger binding
e.g. succinic dehydrogenase Tinoco---p417-8

a) \(-\text{OOC-CH}_2\text{-CH}_2\text{-COO}^- \xrightleftharpoons{\text{S-H}_1} \text{-OOC-CH=CH-COO}^-\)

succinate \hspace{1cm} fumarate

if add malonate: \(-\text{OOC-CH}_2\text{-COO}^-\)
has similar shape, 2 \(-\text{COO}^-\) groups – binds active site but cannot dehydrogenate (not form C=C)
so stays on enzyme: EI forms, ties up E
inhibit reaction of succinate: less ES
like case (a), uses same site — Competitive inhibition

b) Triosephosphate dehydrogenase - Cys in active site

if alkylate: \(\text{TPD-S-H + I-CH}_2\text{CNH}_2 \rightarrow \text{TPD-S-CH}_2\text{C-NH}_2\)

This enzyme now lost to reaction inhibition (no reversal)
but \(K_M\) same, \(\nu_{max}\) reduced (recall slope \(K_m/\nu_{max}\))
⇒ like case (c), sort of kills off the enzyme
– non-competitive \(\alpha, \alpha' > 1\)
see figure above
Allosterism → binding substrate to one site/subunit affect binding/release at 2nd or 3rd ...
e.g. hemoglobin – 4 subunits, each bind O₂ → cooperate

MWC model → each subunit has 2 forms
different binding efficiency

R – relaxed – ○ - binds better
T – tense - □ - no substrate:  \( L = \frac{[T_0]}{[R_0]} \) equilibrium

Solve mechanism use pre-equilibrium:
\[
\nu = \frac{v_{max} \gamma(1+\gamma)}{(1+\gamma)^2+L} \\
\gamma = \frac{[S]}{K_R} \\
L = \frac{[T_0]}{[R_0]}
\]

\( L = 0 \) → no T  →  \( \nu = \frac{v_{max}\gamma}{1+\gamma} \) this is M–M

L increase → sigmoidal shape in rate vs. [S]
This is like inhibitor, reduces amount of R available to bind S
→ binding S convert T → R  ⇒  max rate constant
analogous to increasing enzyme \([E_0]\), only R binds

\( K_R \) increase: denom \( \rightarrow (1+L) \sim 1 \) and \( \nu = \frac{v_{max}\gamma = v_{max}[S]}{K_R} \)
So inc. \( K_R \) dec. rate – it dissociates E-S complex