Lecture - Enzyme Kinetics 11.02.2014

Enzyme Kinetics

Bimolecular reaction $A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$ $\quad k_1\ (M^{-1}s^{-1})$

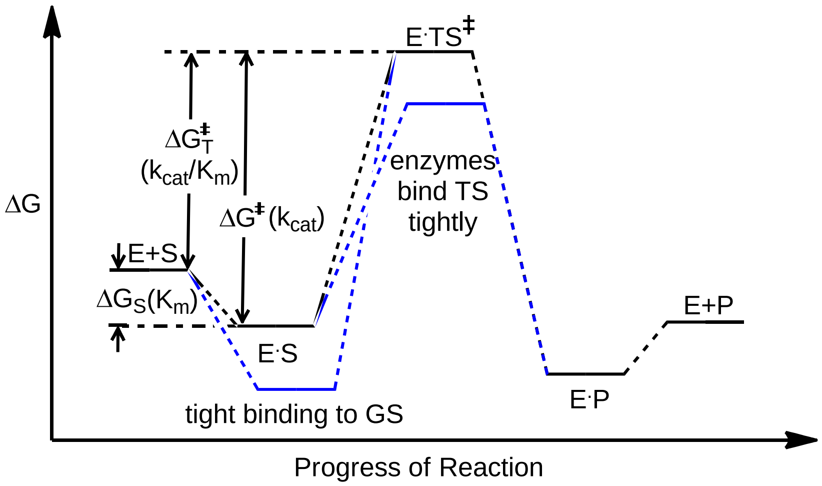
$$\frac{dv}{dt} = k_1 [A] [B] \quad \text{at low conversion}$$

$$E + S \rightleftharpoons E{\cdot}S \rightleftharpoons E{\cdot}P \rightleftharpoons E + P$$

$E{\cdot}S$ ↓ Michaelis Complex

Low conversion $[P] << [S]$

simplify to $E + S \overset{K_m}{\rightleftharpoons} E{\cdot}S \xrightarrow{k_{cat}} E + P$



Enzymes evolve maximal affinity to TS‡

K_m ~ [physiological conc. of substrates] mM-uM

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

$$v = \frac{dP}{dt} = k_{cat} [E{\cdot}S]$$

$$\frac{d[E{\cdot}S]}{dt} = k_1 [E] [S] - k_{-1}[E{\cdot}S] - k_{cat} [E{\cdot}S]$$

$$[E_T] = [E] + [E{\cdot}S]$$

at steady state $\frac{d[E{\cdot}S]}{dt} \sim 0$

$$\left\{ \begin{array}{l} \frac{d[E{\cdot}S]}{dt} = k_1 [E] [S] - k_{-1}[E{\cdot}S] - k_{cat} [E{\cdot}S] = 0 \\ [E] = [E_T] - [E{\cdot}S] \end{array} \right.$$

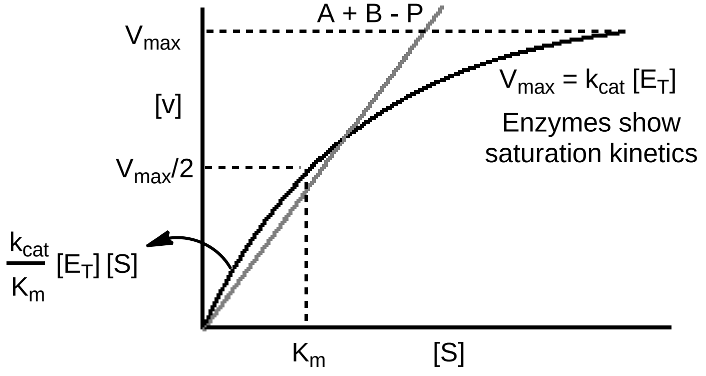
$$\Rightarrow k_1 ([E_T] - [E{\cdot}S]) [S] - k_{-1}[E{\cdot}S] - k_{cat} [E{\cdot}S] = 0$$

$$[E{\cdot}S] = \frac{k_1 [E_T] [S]}{k_{-1} + k_{cat} + k_1 [S]} = \frac{[E_T] [S]}{[S] + \left(\frac{k_{-1} + k_{cat}}{k_1}\right)}$$

define $K_m = \frac{k_{-1} + k_{cat}}{k_1}$ $\quad$ So if $k_{-1} >> k_{cat}$, $K_m \sim K_s = k_{-1}/k_1$

$$[E{\cdot}S] = \frac{[E_T] [S]}{[S] + K_m}$$

$$\boxed{v = \frac{dP}{dt} = k_{cat} [E{\cdot}S] = \frac{k_{cat} [E_T] [S]}{[S] + K_m}}$$



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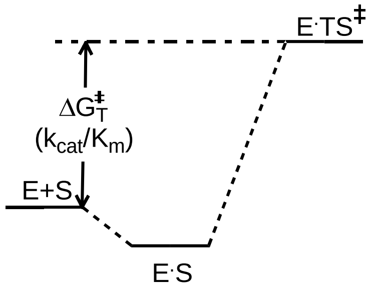
$$\boxed{v = \frac{dP}{dt} = k_{cat}[E{\cdot}S] = \frac{k_{cat}[E_T][S]}{[S] + K_m}}$$

(1) $[S] \gg K_m$ $\quad v = \frac{dP}{dt} = k_{cat}[E_T] = V_{max}$ Saturation Kinetics

(2) at low [S], $[S] \ll K_m$

$$v = \frac{dP}{dt} = \frac{k_{cat}}{K_m}[E_T][S]$$

$\frac{k_{cat}}{K_m}$: catalytic efficiency $(M^{-1}s^{-1})$



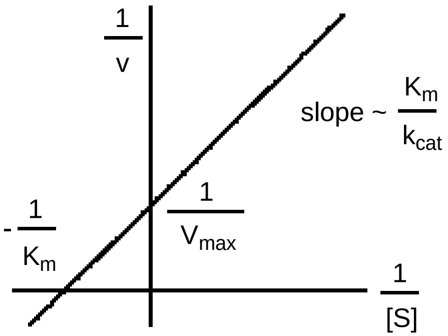
(3) if $[S] = K_m$ $\quad v = \frac{dP}{dt} = \frac{k_{cat}[E_T]}{dt} = \frac{V_{max}}{2}$

Lineweaver-Burk Plot

$$v = \frac{dP}{dt} = k_{cat}[E{\cdot}S] = \frac{k_{cat}[E_T][S]}{[S] + K_m} \qquad V_{max} = k_{cat}[E_T]$$

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

$$\boxed{\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{k_{cat}} \cdot \frac{1}{[S]}}$$



Competitive Inhibition

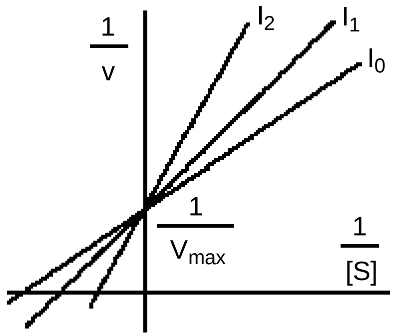
$$\begin{tikzcd} E + S \arrow[r, shift left, "K_m"] \arrow[d, shift left, "{[\,I\,],\ K_i}"] & E{\cdot}S \arrow[l, shift left] \arrow[r, "k_{cat}"] & E + P \\ E{\cdot}I \arrow[u, shift left] & & \end{tikzcd}$$

S and I bind same or overlapping site

$$v = \frac{k_{cat}[E_T][S]}{[S] + K_m\left(1 + \frac{[\,I\,]}{K_i}\right)}$$

V_{max} is unchanged (can saturate with substrate)

K_m reduced by $\left(1 + \frac{[\,I\,]}{K_i}\right)$

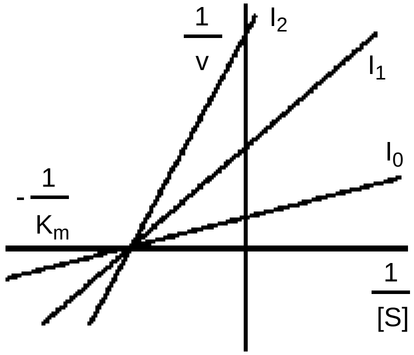


Noncompetitive Inhibition

$$\begin{tikzcd} E + S \arrow[r, shift left, "K_m"] \arrow[d, shift left] & E{\cdot}S \arrow[l, shift left] \arrow[r, "k_{cat}"] \arrow[d, shift left, "{[\,I\,],\ K_i}"] & E + P \\ E{\cdot}I \arrow[u, shift left, "{[\,I\,],\ K_i}"] \arrow[r, shift left, "{[S],\ K_m}"] & E{\cdot}S{\cdot}I \arrow[l, shift left] \arrow[u, shift left] & \end{tikzcd}$$

$$v = \frac{k_{cat}[E_T][S] \Big/ \left(1 + \frac{[\,I\,]}{K_i}\right)}{[S] + K_m}$$

K_m is unchanged



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Rate constants

diffusion controlled rate constants for small molecules

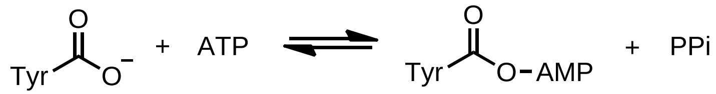
$\mathrm{A + B} \rightleftharpoons \mathrm{P} \quad 10^9 \sim 10^{10}\ \mathrm{M^{-1}s^{-1}}$

	k_1 ($M^{-1}s^{-1}$)	k_{-1} (s^{-1})
ribonuclease + Up	7.8×10^7	1×10^4
Tyr-tRNA synthetase + $tRNA^{Tyr}$	2.0×10^8	1.5

	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (M)
acetylcholine estalase	1.6×10^8	1.4×10^4	9×10^{-5}
catalase	4.0×10^3	4×10^3	1
β–lactamase	1.0×10^8	2×10^3	2×10^{-5}

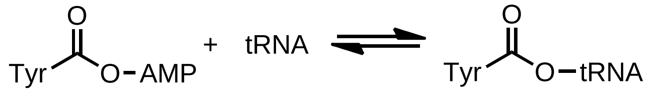
Tyrosyl tRNA synthetase

(1)



$\mathrm{Tyr{-}C({=}O){-}O^-} + \mathrm{ATP} \rightleftharpoons \mathrm{Tyr{-}C({=}O){-}O{-}AMP} + \mathrm{PPi}$

(2)

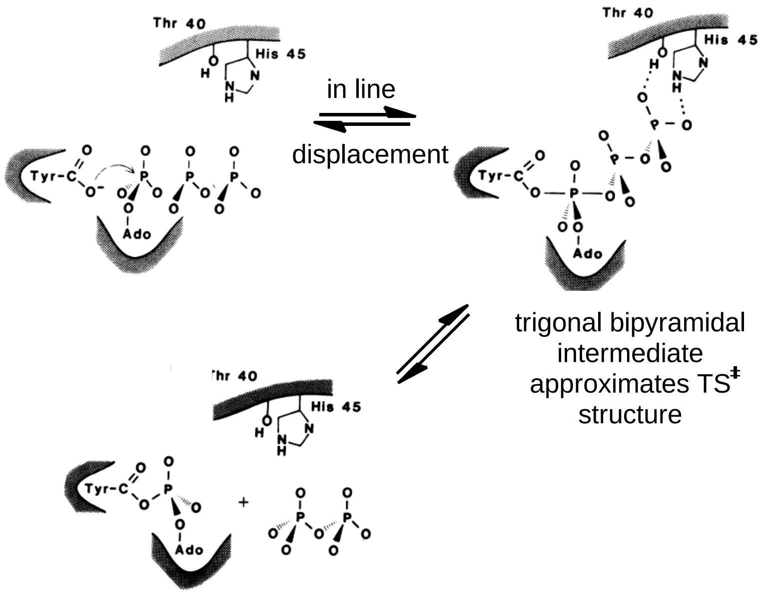


$\mathrm{Tyr{-}C({=}O){-}O{-}AMP} + \mathrm{tRNA} \rightleftharpoons \mathrm{Tyr{-}C({=}O){-}O{-}tRNA}$

$$\begin{tikzcd}
 & E{\cdot}ATP \arrow[dr, shift left, "k_{t'}\,[T]"] \arrow[dl, shift left, "k_{-a}"] & & & \\
E \arrow[ur, shift left, "k_a\,[A]"] \arrow[dr, shift left, "k_t\,[T]"] & & E{\cdot}Tyr{\cdot}ATP \arrow[ul, shift left, "k_{-t'}"] \arrow[dl, shift left, "k_{-a'}"] \arrow[r, shift left, "k_3"] & E{\cdot}Tyr{-}AMP{\cdot}PPi \arrow[l, shift left, "k_{-3}"] \arrow[r, shift left, "k_{app}"] & E{\cdot}Tyr{-}AMP + PPi \arrow[l, shift left, "k_{-app}"] \\
 & E{\cdot}Tyr \arrow[ul, shift left, "k_{-t}"] \arrow[ur, shift left, "k_{a'}\,[A]"] & & &
\end{tikzcd}$$

rate-limiting step: $k_3 \sim k_{cat}$

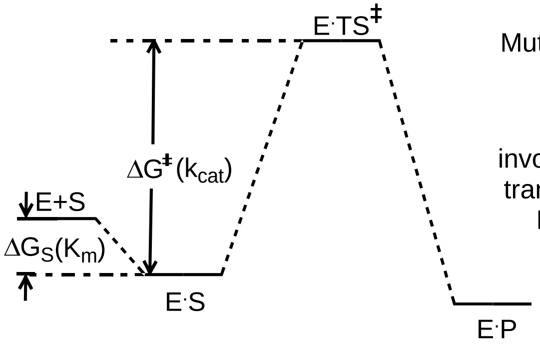
$$K_m \sim K_s = \frac{k_{-t'}}{k_{t'}} \quad \text{or} \quad \frac{k_{-a'}}{k_{a'}}$$



trigonal bipyramidal intermediate approximates TS‡ structure

Leatherbarrow, R. J., Fersht, A. R., and Winter, G. (1985) PNAS 82, 7840-7844.

	k_{cat} (s^{-1})	K_s (Tyr) (uM)	K_s (ATP) (mM)
wt TyrRS	38	12	4.7
His45Gly	0.16	10	1.2
Thr40Ala	0.0055	8	3.8



Mutations largely affect k_{cat} but not K_m

⇓

involved in stabilizing the transitition state, but not Michaelis complex

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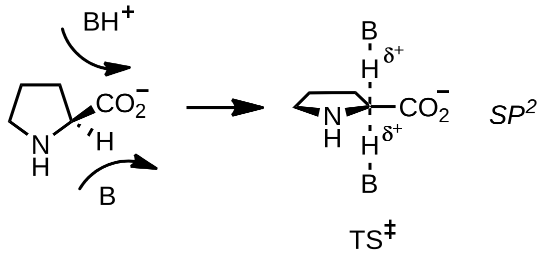
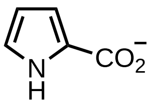
Design of transition state analog inhibitors

Proline Racemase

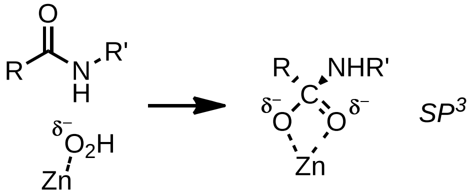


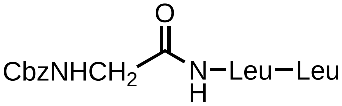
inhibitor



bind 160 times tighter than Pro

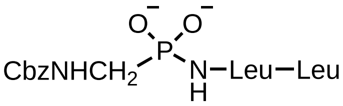
Thermolysin





$K_m = 26$ mM

inhibitor



$K_i = 9.1$ nM