Michaelis-Menton kinetics

The rate of an enzyme catalyzed reaction in which substrate S is converted into products P depends on the concentration of the enzyme E even though the enzyme does not undergo any net change.

$$E + S \underset{k'_{off}}{\overset{k_{on}}{\leftrightarrow}} ES \xrightarrow{k_{cat}} P + E$$

Michaelis-Menton rate equations

$$E + S \underset{k_{off}}{\overset{k_{on}}{\leftrightarrow}} ES \xrightarrow{k_{cat}} P + E$$

$$\frac{d[S]}{dt} = -k_{on}[E][S] + k_{off}[ES]$$

$$\frac{d[ES]}{dt} = k_{on}[E][S] - k_{off}[ES] - k_{cat}[ES]$$

$$\frac{d[P]}{dt} = k_{cat}[ES]$$

Steps in the Michaelis-Menton mechanism

Step 1. Bimolecular formation of the enzyme E and and substrate S:

$E + S \longrightarrow ES$ rate of formation of ES = $k_{on}[E][S]$

Step 2. Unimolecular decomposition of the complex:

$ES \longrightarrow E + S$ rate of decomposition of ES = $-k_{off}[ES]$

Step 3. Formation of products and release from the enzyme:

$ES \longrightarrow P + E$ rate of formation of P = $k_{cat}[ES]$

The rate law of interest is the formation of the product in terms of E and S.

The enzyme substrate complex can be eliminated

The enzyme substrate complex is formed transiently and can be approximated using the steady state approximation.

$$\frac{d[ES]}{dt} = k_{on}[E][S] - k_{off}[ES] - k_{cat}[ES] \approx 0$$

The result of this approximation is

$$[ES] = \frac{k_{on}[E][S]}{k_{off} + k_{cat}}$$

Pseudo-first order Michaelis-Menton kinetics

In an experiment we know the total enzyme concentration $[E]_0$ and not the unbound enzyme [E]. The total concentration of enzyme $[E]_0 = [E] + [ES]$.

$$[ES] = \frac{k_{on}([E]_0 - [ES])[S]}{k_{off} + k_{cat}}$$

which rearranges to

$$[ES]\left(1 + \frac{k_{on}[S]}{k_{off} + k_{cat}}\right) = \frac{k_{on}[E]_0[S]}{k_{off} + k_{cat}}$$

Pseudo-first order Michaelis-Menton kinetics

At this point it is convenient to define the Michaelis constant

$$K_m = \frac{k_{off} + k_{cat}}{k_{on}}$$

and to rearrange the equations as

$$[ES] = \frac{[E]_0[S]}{K_m\left(1 + \frac{[S]}{K_m}\right)} = \frac{[E]_0[S]}{K_m + [S]}$$

Michaelis-Menton parameters

The rate of formation of product can be written

$$\frac{d[P]}{dt} = \frac{k_{cat}[E]_0[S]}{K_m + [S]}$$

where K_m is the Michaelis constant and k_{cat} is the maximum turnover number. We often make the definitions

$$V_0 = \frac{d[P]}{dt} \qquad V_{max} = k_{cat}[E]_0$$

which permit us to write the equation as

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Limiting conditions of enzyme reactivity

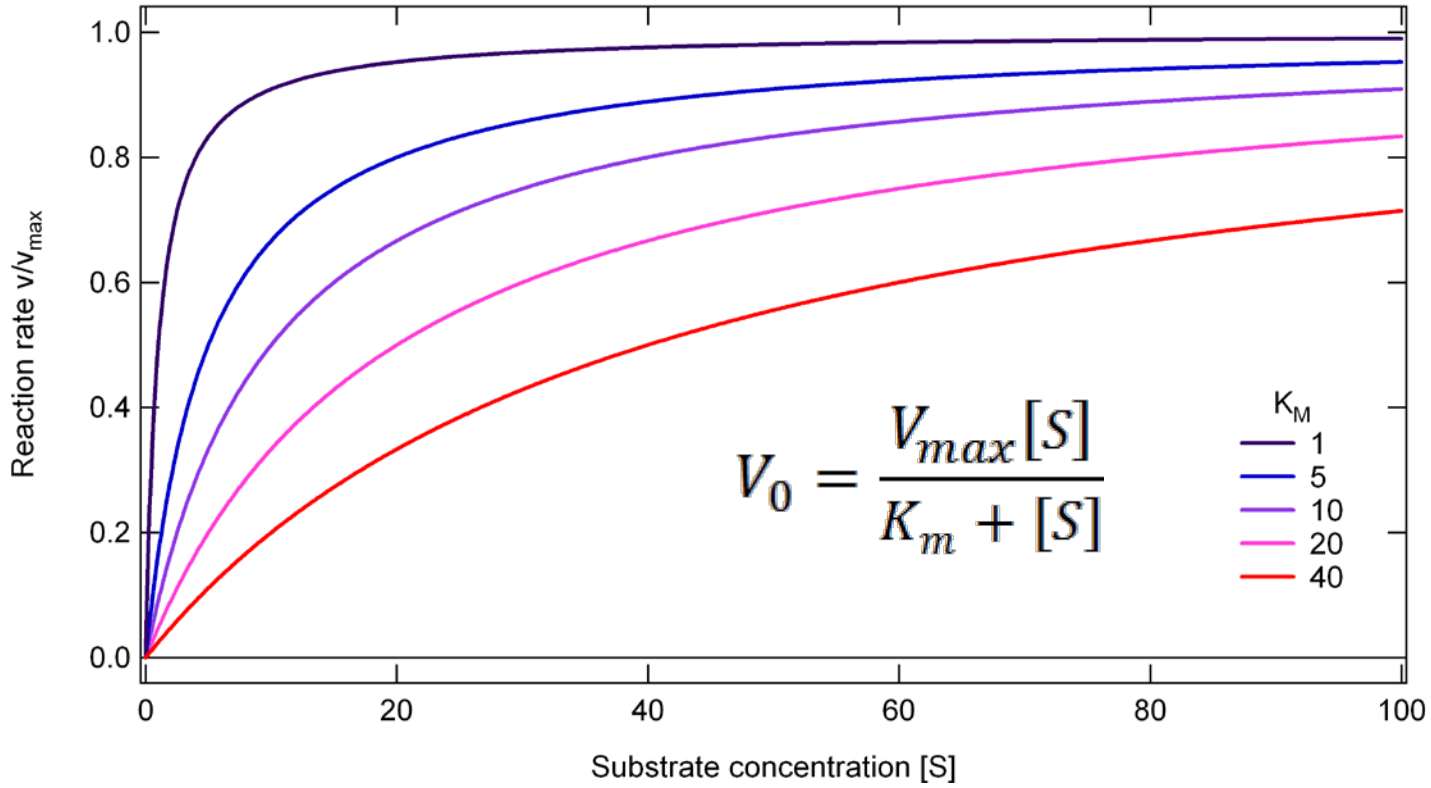
• Maximal rate: If there is excess substrate present the rate is limited by the rate at which the ES complex falls apart. The rate of formation of products is a maximum and $v_{max} = k_{cat}[E]_0$ is called the maximum velocity.

• Second order regime: If $[S] << K_M$ then the rate of formation of products is $d[P]/dt = k_{cat}/K_m\ [E]_0[S]$. The rate depends on [S] as well as $[E]_0$.

• A plot of 1/k yields k_{cat} and K_m but not the rate constants k_{on} and k_{off}. The latter rate constants can be obtained from stopped-flow experiments.

General expression for reaction velocity

Based on the previous analysis the velocity at an arbitrary substrate concentration is:



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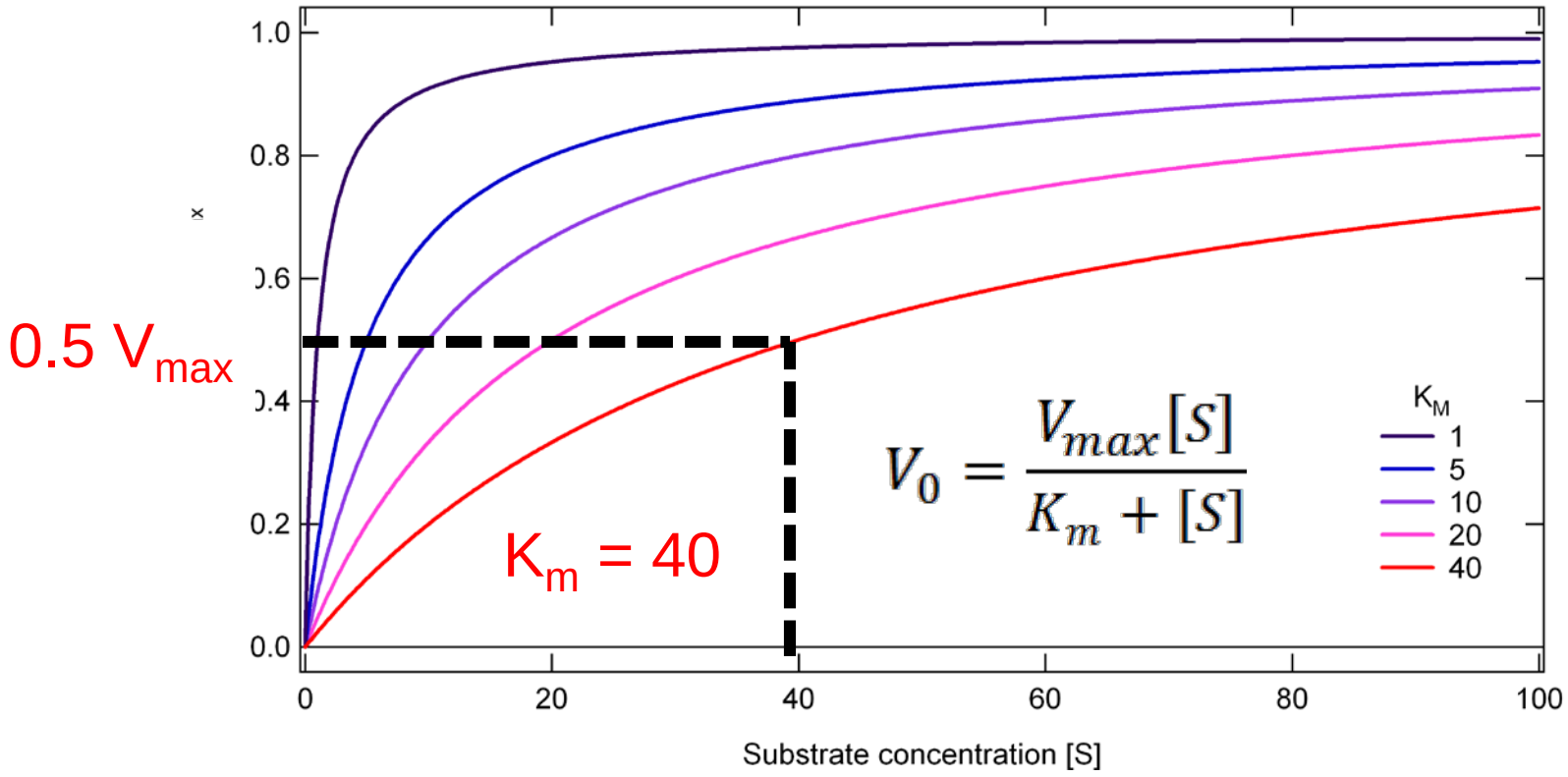


Lineweaver-Burke Plots

- The Michaelis-Menton expression is non-linear.
- The Lineweaver-Burke plot is linearized plot of data.

$$\frac{1}{v} = \frac{K_M + [S]}{[S]\,v_{\max}} = \frac{1}{v_{\max}} + \left(\frac{K_M}{v_{\max}}\right)\frac{1}{[S]}$$

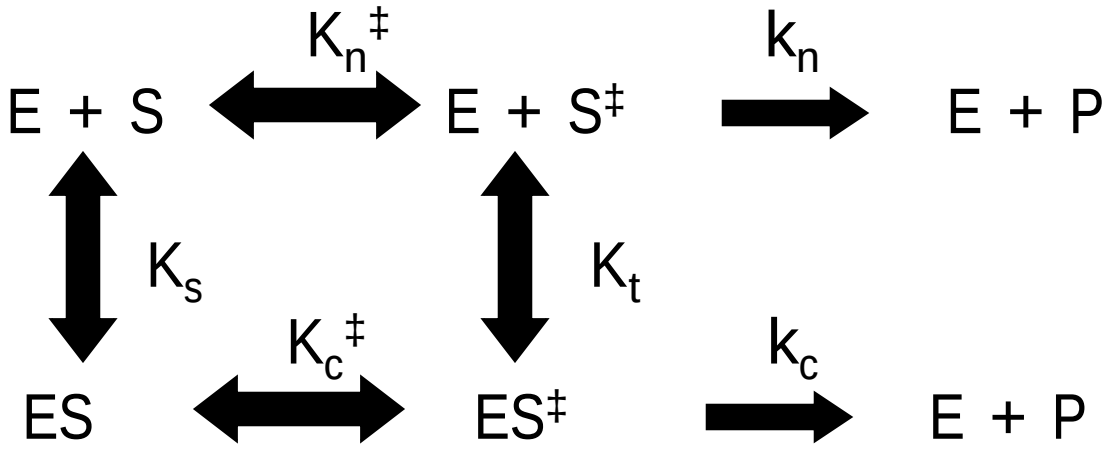
- This expression has the form of an equation for a line:

y = intercept + slope x

- Such plots are not necessary today with common non-linear fitting programs.

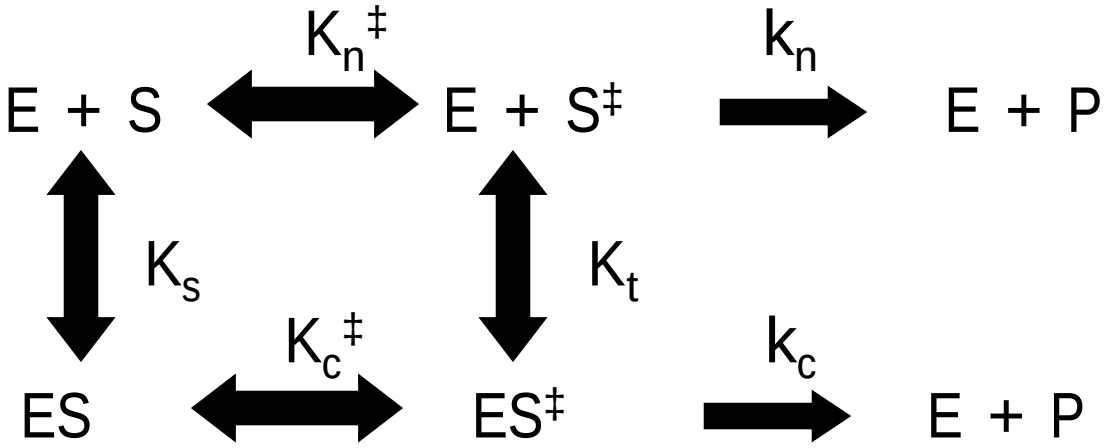
Transition State Stabilization

The original idea of the enzyme having maximum complementarity to the TS was put forward by Linus Pauling in 1946. It wasn't until the early 70's that the idea was put on a more solid grounding. As put forward by Lienhard and Wolfenden the idea is as follows:



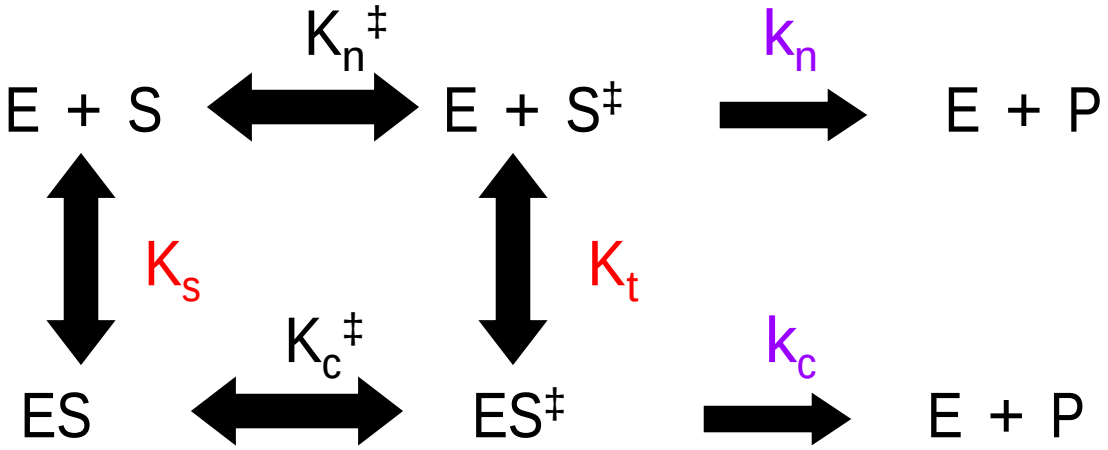
Transition State Stabilization

Defining the equilibrium constants as association constants:
$K_n^{\ddagger} = [S^{\ddagger}]/[S]$, $K_c^{\ddagger} = [ES^{\ddagger}]/[ES]$, $K_t = [ES^{\ddagger}]/[E][S^{\ddagger}]$
from TS theory: $\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$ and $k_{obs} = (k_B T/h)e^{-\Delta G^{\ddagger}/RT}$
Thus, $k_n = (k_B T/h)K_n^{\ddagger}$ and $k_c = (k_B T/h)K_c^{\ddagger}$
where c means catalyzed and n means uncatalyzed.



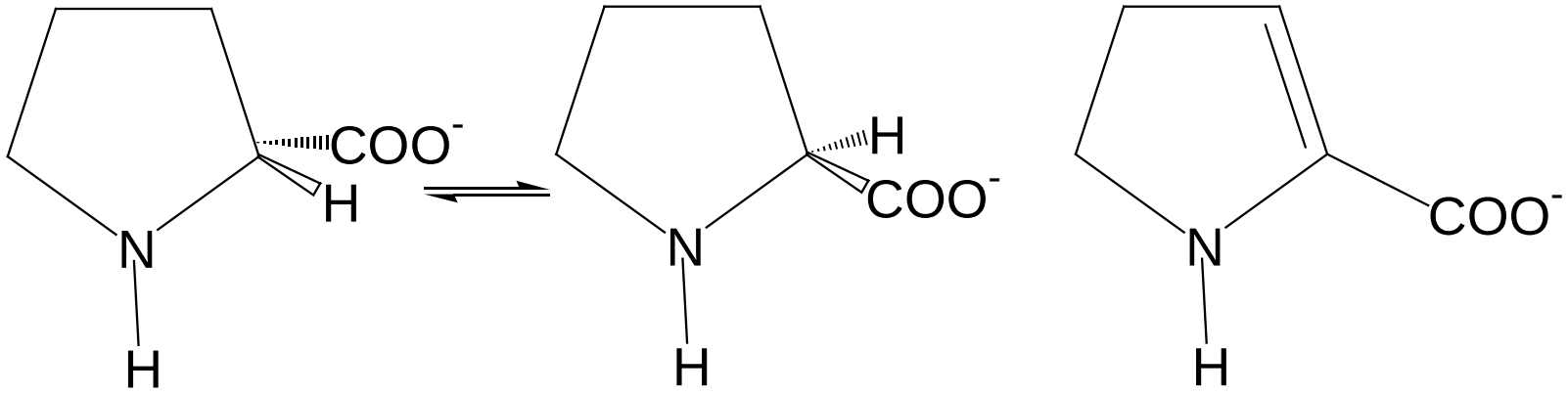
Transition State Stabilization

From the scheme you can see that $K_s\ K_c^{\ddagger} = K_n^{\ddagger}\ K_t$
hence $K_t/K_s = K_c^{\ddagger}/K_n^{\ddagger}$ however, $k_c/k_n = K_c^{\ddagger}/K_n^{\ddagger}$
Therefore the observed rate enhancement
$k_c/k_n = K_t/K_s >> 1$
Therefore the transition state geometry $S^{\ddagger}$ must bind more tightly than the substrate S in its equilibrium geometry!



Transition State Analogs

The transition state stabilization hypothesis was tested by designing so-called transition state analogs, molecules which mimick the real TS as closely as possible. One of the first enzymes examined was proline racemase:



The compound on the right is a planar TS state analog. This molecule was found to be a good inhibitor, with K_i some two orders of magnitude smaller than K_m.