Enzyme Kinetics: The study of reaction rates.

For the *1st-order* reaction $S \longrightarrow P$ the Velocity (V) is:

$$V = \frac{d[P]}{dt} = -\frac{d[S]}{dt} \approx \frac{\Delta P}{\Delta t} = \frac{moles/L}{\sec}$$

In a *1st*-order reaction $V = k_1[S]$

k_1 is a rate constant with units $1/s = s^{-1}$; V has units of moles per litre per second.

In a reversible *1st–order* reaction, $S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$

the **net** reaction rate V is $V = k_1[S] - k_{-1}[P]$

At equilibrium, $V = 0$ and, $k_1[S]_{eq} = k_{-1}[P]_{eq}$ or

$$\frac{[P]_{eq}}{[S]_{eq}} = \frac{k_1}{k_{-1}} = K_{eq}$$

In a *2nd*–order reaction, $A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$

the net rate of reaction is $V = k_1[A][B] - k_{-1}[C]$

The forward rate constant k_1 has the units $\frac{litres}{moles \bullet \sec}$

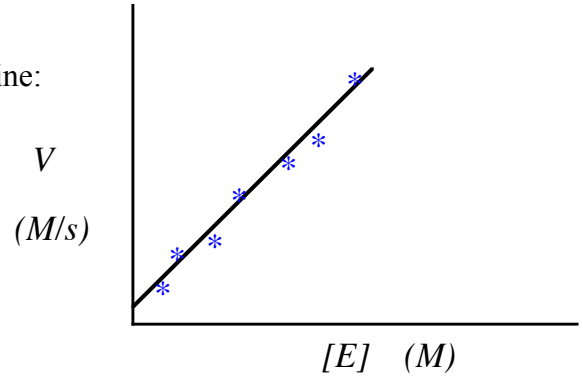
So $\frac{litre}{moles \bullet \sec} \bullet \frac{moles}{litre} \bullet \frac{moles}{litre} = \frac{moles}{litre \bullet \sec}$

Factors affecting Enzyme-Catalysed Reactions:

1. **Enzyme Concentration**

Usually, $[E] << [S]$ and $V \alpha [E]$

If $[S]$ is the same in each assay, a graph of V *vs*. $[E]$ is a straight line:



2. Substrate Concentration:

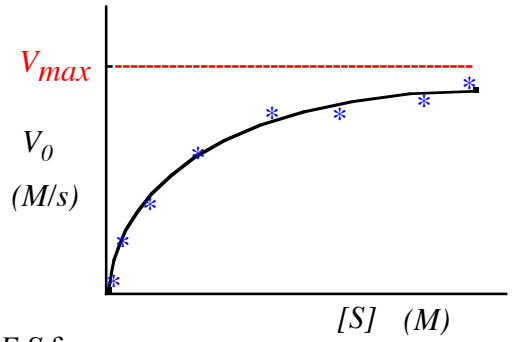
As $S \longrightarrow P$, $[S]$ decreases. However, when $[E] << [S]$, $[S]$ will change only a little at the beginning of the reaction and can be considered "*constant*".

V_0 = reaction rate at $t = 0$. $= \frac{d[P]_0}{dt} = -\frac{d[S]_0}{dt} \approx \frac{\Delta P}{\Delta t}$

At low $[S]$, $V_0 \alpha [S]$.

At high $[S]$, V_0 is independent of $[S]$, and $V_0 = V_{max}$.

This curve is **hyperbolic**.



This was the first hint that $E{\cdot}S$ forms.

At low [S], most of the E is unbound. As [S] increases, more and more $E{\cdot}S$ forms and V_0 increases.

At high [S], all the E is present as $E{\cdot}S$ so adding S cannot increase V_0. The E is **saturated** with S at V_{max}.

Note: V_{max} will be different for different [E].

We will now present an equation to describe this curve. It is based on the following mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

k_1, k_{-1} *etc*. are rate constants.

At $t = 0$, $[P] = 0$ so there is no back reaction and $k_{-2} \sim 0$, and:

$$V_0 = \frac{k_2[ES][S]}{[S] + \frac{k_{-1} + k_2}{k_1}}.$$

We define the Michaelis Constant K_m

$$\frac{k_{-1} + k_2}{k_1} = K_m$$

When the Enzyme is **saturated**, $[E{\cdot}S] = [E_{tot}]$ and

$$V_0 = V_{\max} = k_2[E_{tot}]$$

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

This is the **Michaelis-Menton Equation**.

It describes the relationship between V_0 and $[S]$;

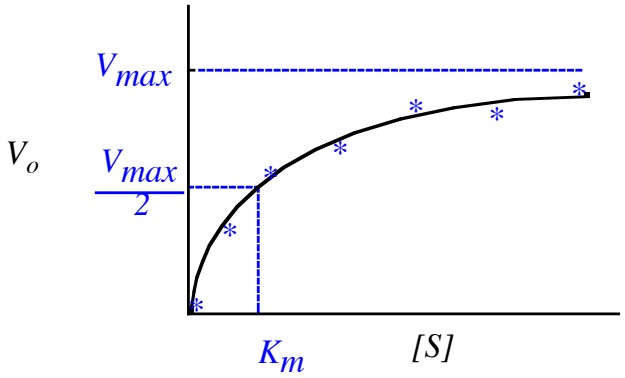
V_{max} and K_m are characteristics of particular enzymes.

V_{max} is the maximum rate of reaction at a particular $[E_{tot}]$.

When $V_0 = \frac{1}{2}V_{\max}$ then $V_0 = \frac{V_{\max}}{2} = \frac{V_{\max}[S]}{K_m + [S]}$ and

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \quad \text{and} \quad K_m = [S] \ !$$

So K_m is numerically equal to the $[S]$ at which $V_0 = \frac{V_{\max}}{2}$



Recall that $K_m = \frac{k_{-1} + k_2}{k_1}$. Quite often $k_2 << k_{-1}$

So $K_m \approx \frac{k_{-1}}{k_1} = K_S = \frac{[E][S]}{[E \cdot S]}$

which is the **equilibrium Enzyme-Substrate dissociation constant**, a measure of the affinity of *S* for *E*.

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

Big K_S or K_m means weak binding. Small K_S or K_m means strong binding.

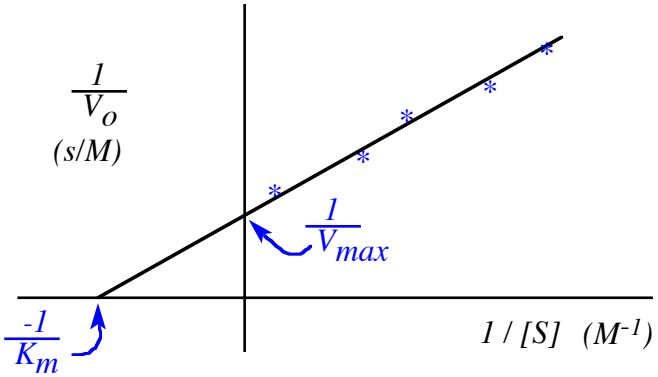
One way to determine K_m and V_{max} is by non-linear least-squares fitting of the Michaelis-Menton equation to measured data.

Another way is a reciprocal plot of the MM equation.

The inverse of $V_0 = \frac{V_{max}[S]}{[S] + K_m}$ is $\frac{1}{V_0} = \frac{K_m}{V_{max}} \bullet \frac{1}{[S]} + \frac{1}{V_{max}}$

$Y = m \cdot X + b$

$Slope = \frac{K_m}{V_{max}}$ $\quad Y - intercept = \frac{1}{V_{max}}$ $\quad X - intercept = -\frac{1}{K_m}$



Recall that V_{max} depends on the [E] and when the E is saturated with S

$$V_{\max} = k_2[E_{tot}]$$

$$k_2 = \frac{V_{\max}}{E_{tot}} = k_{cat}$$

So k_{cat} is an E-parameter that is independent of [E] and reports how fast an E works.

e.g. At pH 7, 35°C carbonic anhydrase hydrates $4x10^5$ moles of CO_2 yielding $4x10^5$ moles of HCO_3^- per mole of enzyme per second – k_2 is a 1st-order rate constant.

TABLE 5.1 Examples of catalytic constants

Enzyme	$k_{cat}(s^{-1})$*
Papain	10
Ribonuclease	10^2
Carboxypeptidase	10^2
Trypsin	10^2 (to 10^3)
Acetylcholinesterase	10^3
Kinases	10^3
Dehydrogenases	10^3
Transaminases	10^3
Carbonic anhydrase	10^6
Superoxide dismutase	10^6
Catalase	10^7

*The catalytic constants are given only as orders of magnitude.

Table 5-1 Principles of Biochemistry, 4/e

k_{cat} can be used for non-M-M enzymes as well.

Since: $V_0 = \frac{k_{cat}[E_{tot}][S]}{[S]+K_m}$ when [S] is << K_m $V_0 \cong \frac{k_{cat}[E_{tot}][S]}{K_m}$

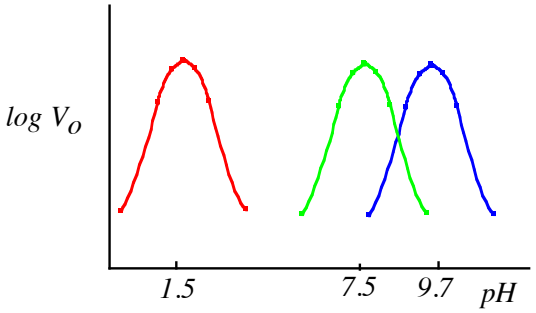
$\frac{k_{cat}}{K_m}$ is a 2^{nd} –order rate constant that measures how fast E and S react. The units are $\frac{litres}{moles \bullet \sec}$

The fastest that two molecules can diffuse together is about 10^9 -$10^{11}M^{-1}\cdot s^{-1}$ so this puts a limit on how fast the reaction can go.

$\frac{k_{cat}}{K_m}$ ranges from 0.36 to 2.4 X 10^8 $M^{-1}\cdot s^{-1}$.

3. Many enzymes exhibit an optimum pH at which maximum activity occurs.

Pepsin = 1.5
Carboxypeptidase A = 7.5
Arginase = 9.7



Why?

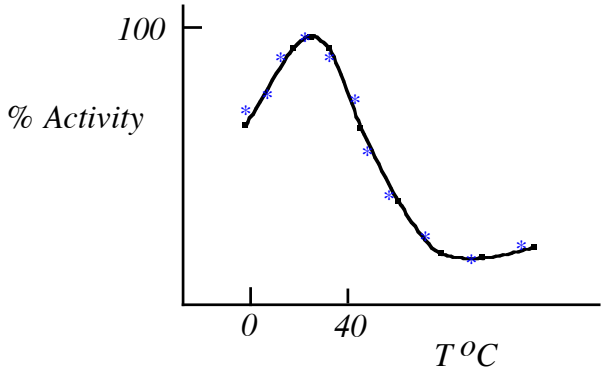
i. pH may change the ionization states of AA in the active site.

ii. pH may change the ionization state of *S*.

iii. pH may denature the *E*.

4. Temperature dependence is determined by: $k = \frac{k_B \bullet T}{h} \bullet e^{(\frac{-\Delta G^{\dagger}}{R \bullet T})}$

and by denaturation at high and low *T*.



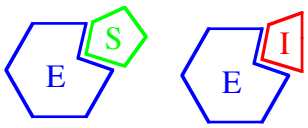
5. Inhibitors

i. Some are foreign, *e.g.* poisons, antibiotics, pesticides, herbicides.

ii. Some are naturally present for regulation of enzymatic activity.

Competitive Inhibitors (I)

I is similar in structure to **S** and binds competitively to the **same site on the E** as **S**.

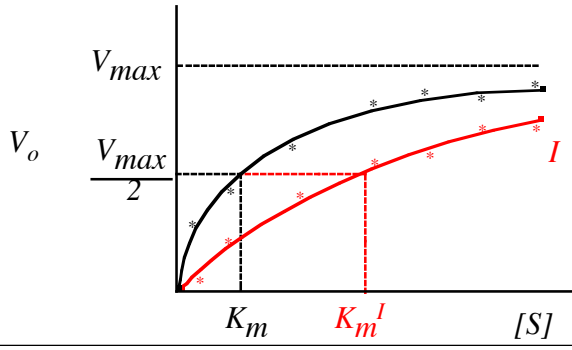


e.g. malonic acid, **$HOOCCH_2COOH$**, is an inhibitor of succinate dehydrogenase: Succinic acid = **$HOOCCH_2CH_2COOH$**

How does **I** affect the V_o?

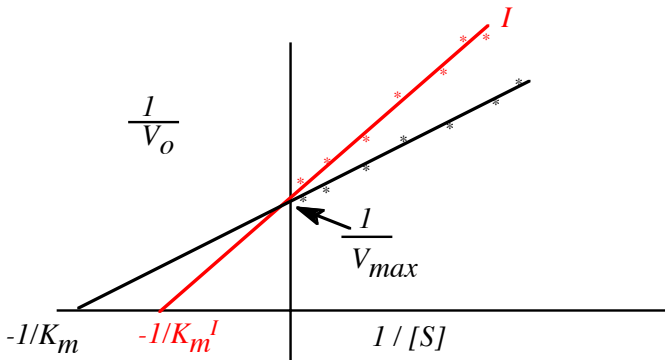
At high [*S*] the *I* is displaced from *E* so V_{max} is unchanged.

$$E + S + I \rightleftharpoons ES + EI$$



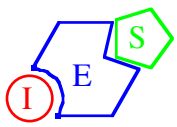
But K_m is **apparently** increased because it takes much more *S* to reach V_{max}.

Recall that K_m is the *apparent* affinity of *E* for *S*.



Non-Competitive Inhibitors

I binds at a site distinct from the substrate site, usually an **allosteric** site. *Allos* - Greek - "*other*" *Stereos* - "*shape*".



$$E + S \rightleftharpoons ES \rightarrow E + P$$

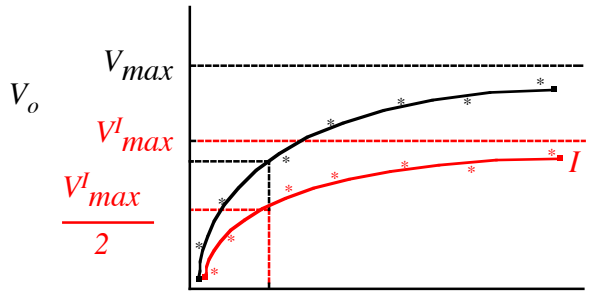
$$+ \quad I \qquad + \quad I$$

$$K_I \qquad K_I$$

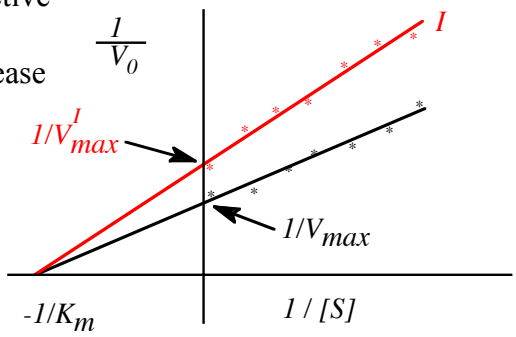
$$EI + S \rightleftharpoons EIS \not\rightarrow$$

It may bind to free *E* or to *ES*. Once bound, it will prevent *P* formation.

If the binding affinities of *I* to *E* and ES are identical, there will be no effect on K_m.



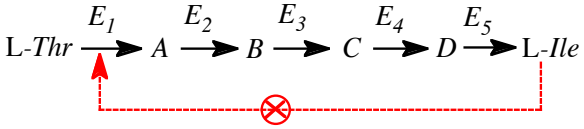
But since the *I* decreases active $[E_{tot}]$ then V_{max} must decrease $(= k_{cat}[E_{tot}])$.



So graphical analysis can give important information about the nature of the inhibitor and its mechanism of action.

Regulatory Enzymes

A metabolic pathway is one in which the product of the 1st Enzyme is a substrate for the 2nd Enzyme *etc*.



Often regulation occurs at the beginning of the path to prevent waste.

E_1 is *Threonine dehydratase*. It is a key regulatory enzyme in the pathway and is inhibited by *L*-Ile.

End-Product Inhibition / Feedback Inhibition

The regulation shown above is called **heterotropic** because the enzyme is regulated by molecules that are not *S* or *P* of the enzyme being regulated.

If *S* or *P* **modulate** the activity of their own enzyme the regulation is called **homotropic**.

Directed Overflow Regulation

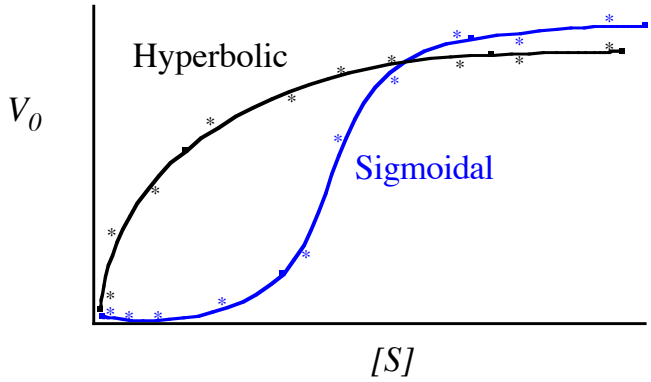
CTP is synthesized from Asp in 7 steps. CTP feedback inhibits its own synthesis by inhibiting the first step of the pathway.

However, when demand is low, CTP can still build up and it is eliminated by degradation to uracil followed by secretion.

Allosteric Enzymes

The V_0 *vs* [*S*] plot is **Sigmoidal** whereas MM plots are **Hyperbolic**.

The enzyme will be very sensitive to [S] over a narrow range and behaves like an on-off switch.



1/V_0 vs 1/[S] curves are **not** linear. It is not proper to speak of K_m

so $S_{0.5}$ is used for [S] needed to reach *1/2* V_{max}.

1. Allosteric enzymes are usually oligomeric, multi-subunit complexes. *i.e.* They have quaternary structure.

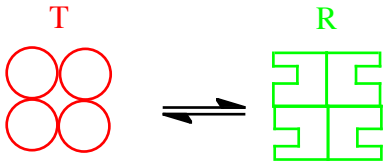
2. They often exhibit **cooperativity**. The binding of ***S*** to one active site of the enzyme makes the binding of subsequent ***S*** easier. How?

3. Each subunit can exist in 2 conformations:

Low affinity - T-state (taut)

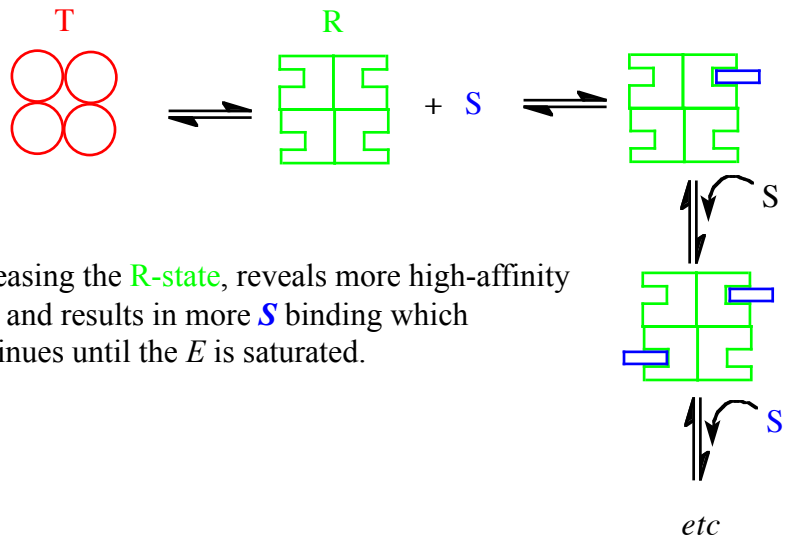
High affinity - R-state (relaxed)

Without **S**, the equilibrium favours T and the affinity of **S** for E is low.



Lots Little

Binding of ***S*** stabilizes the R-state, pulling the equilibrium to the high affinity form simply by Le Chatelier's Principle:

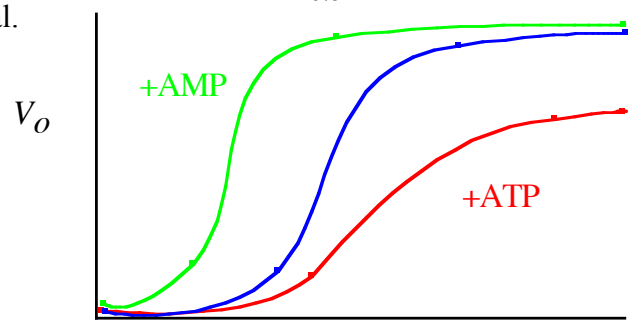


Increasing the R-state, reveals more high-affinity sites and results in more ***S*** binding which continues until the *E* is saturated.

This can be shown to produce sigmoidal V_0 *vs.* [*S*] graphs.

Inhibitors stabilize the **T-state**, increasing $S_{0.5}$, and making the curve less sigmoidal. *e.g.* **ATP** and **citrate** inhibit *phosphofructokinase*.

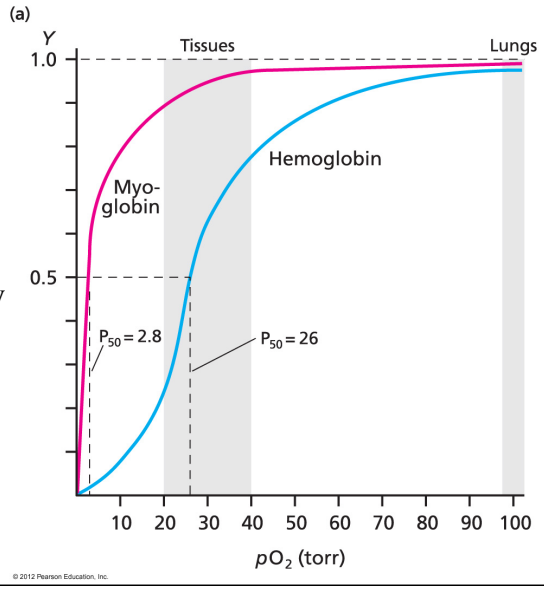
Activators stabilize the **R-state**, decreasing $S_{0.5}$, and making the curve more sigmoidal.



E.g. **AMP** increases PFK activity. *[Fructose-6 phosphate]*

Such inhibitors and activators do not bind at the substrate binding site. They bind at other sites and influence the conformation of the protein.

Hemoglobin binds O_2 cooperatively. This allows it to respond to changes in O_2 demand by different tissues.



O_2 binding is also inhibited allosterically by 2,3-bisphosphoglycerate.

Because fetal hemoglobin has a lower affinity for BPG it has a higher affinity for O_2. This permits the fetus to extract O_2 from the mother's blood.

Covalent Regulation

e.g. Glycogen phosphorylase is activated by phosphorylation of Ser.

This is reversible by a *phosphatase* that removes the phosphate.

Other possibilities: AMP, UMP, methyls, ADP ribose, sugars *etc*. can be added and removed.

In cancer cells, addition of N-acetyl-Glucose to Ser-529 of *phosphofructokinase 1* inhibits the enzyme slowing down glycolysis and speeding up the pentose phosphate pathway.

This permits cells to make more biosynthetic precursors and NADPH allowing faster growth. These changes permit much faster metabolic changes than are possible by gene regulation.

Irreversible Inhibitors

Usually they form a covalent bond with an active site AA.

e.g. Penicillin binds to the active site Ser in *transpeptidase*, an enzyme involved in bacterial cell wall synthesis.

e.g. Diisopropylfluorophosphate binds to Ser-195 in *chymotrypsin*.

Zymogens: Enzyme activation by proteolytic cleavage.

e.g. The pancreas produces inactive *trypsinogen*, *chymotrypsinogen*, *proelastase*, and *procarboxypeptidase* to prevent digestion of the pancreas.

In the gut, a duodenal enzyme ***enteropeptidase*** activates trypsin by removing AA 1-6 of *trypsinogen* producing active *trypsin*.

Then…