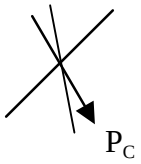
Enzyme Catalysis

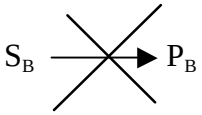
Readings: SAB, pp. 745-752

Enzymes are biological catalysts, consisting of proteins, that greatly accelerate reaction rates and do so with exquisite **specificity**. That is, a chemical reaction involving one reactant goes much faster, but other chemical reactions involving the same reactant or the same chemical reaction performed on an analogous reactant are sometimes largely unaffected.

$S_A \xrightarrow{E} P_A$ (accelerated)



(not accelerated)

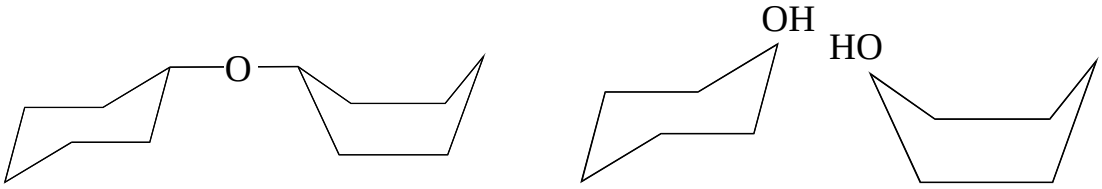


(not accelerated)

The reactant in an enzymatic process is called a <u>substrate</u>.

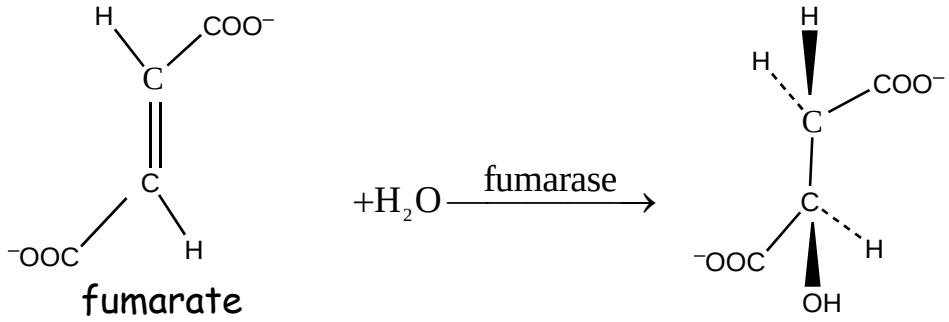
<u>Examples</u>:

1) Sucrose + $H_2O(C_{12}H_{22}O_{11}) \xrightarrow{\text{invertase}}$ glucose ($C_6H_{12}O_6$) + fructose ($C_6H_{12}O_6$).



2) lactose (a C_{12} milk sugar) $\xrightarrow{\text{lactase}}$ glucose + galactose
(lactose intolerant: unable to produce lactose. LactAid and other products contain a form of lactase)

3)



Key features: rate accelerated
product specificity
substrate selectivity [invertase does not work on "splenda" which is chlorinated sucrose.]
stereospecificity

(1) <u>Michaelis-Menten Mechanism</u>

One mechanism that describes the behavior of many enzymes is known as the Michaelis-Menten mechanism.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad (1)$$

$$ES \xrightarrow{k_2} E + P \qquad (2)$$

$$S \rightarrow P \qquad \text{(overall)}$$

In the first step of this mechanism (1), enzyme binds substrate, creating a <u>Michaelis complex</u> (ES). This step is readily reversible. In the second step (2) the substrate is converted to product and is released from the enzyme. Frequently this step may be considered irreversible because the concentrations, [E] and [P], are quite small.

Because [E] is so small and k_2 is usually large, the Michaelis complex (ES) is generally present at low concentration. Treating ES as a steady-state intermediate, we can derive an expression for the reaction rate.

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 .$$

At this point it is convenient to also replace [E], because it is often difficult to measure accurately. This is done by expressing the total enzyme concentration $[E]_0$ as a sum of free enzyme [E] and enzyme bound to substrate [ES].

$[E]_0 = [E] + [ES]$

$[E] = [E]_0 - [ES]$.

Although [ES] is small, the approximation $[E] \approx [E]_0$ is not valid, because $[E]_0$ is usually very small as well. Substituting for [E], one obtains

$$0 = k_1([E]_0 - [ES])[S] - k_{-1}[ES] + k_2[ES] = 0$$

$$[ES]_{SS} = \frac{k_1[E]_0[S]}{k_1[S] + k_{-1} + k_2} = \frac{[E]_0[S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

$$\frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E]_0[S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

This expression is traditionally written

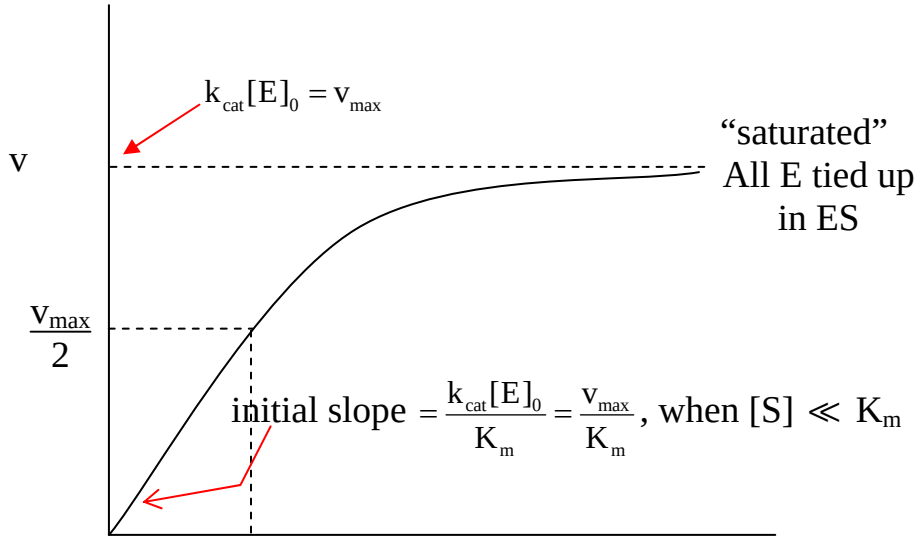
$$v = \left.\frac{d[P]}{dt}\right|_{initial} = \frac{k_{cat}[E]_0[S]}{[S] + K_m}$$ Michaelis-Menten equation

where "v" stands for reaction "velocity", k_2 has been replaced by k_{cat}, and $\frac{k_{-1} + k_2}{k_1}$ has been replaced by K_m, the Michaelis constant.

One typically measures the initial value of v (rate of appearance of P) at a series of initial values of [S].

Two kinds of plots are convenient.

(i) v vs. [S], $v = \left.\frac{d[P]}{dt}\right|_{initial}$



$[S] = K_m[S]$

The "initial slope" must not be confused with the "initial rate." The initial slope is obtained by measuring the initial rate for several reaction mixtures at initial values of [S].

This illustrates two important limits:

(i) $[S] = K_m \rightarrow v \approx \frac{k_{cat}}{K_m}[E]_0[S] \rightarrow$ linear in [S]

(ii) $[S] ? K_m \rightarrow v \approx k_{cat}[E]_0 \rightarrow$ constant with respect to [S].

v_{max} is the maximum reaction rate for a given quantity of enzyme $[E]_0$. It is achieved at a saturating concentration of substrate, at which every enzyme molecule is "busy" carrying out a reaction (ES E + P) and there are no free enzyme molecules "waiting" to find substrate.

k_{cat} is sometimes called the turnover number, because it represents the rate at which each enzyme molecule produces products under these saturating conditions.

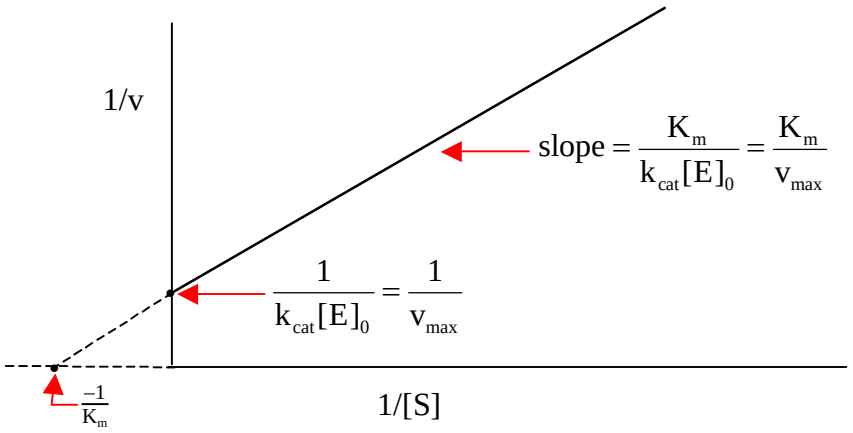
$$\text{Turnover number} = \frac{\text{Maximum number of product molecules formed}}{\text{Number of enzyme molecules}}$$

$$= \frac{\text{Maximum rate of product formation}}{\text{Total concentration of enzyme}} = \frac{k_{max}}{[E]_0} = \frac{k_{cat}[E]_0}{[E]_0} = k_{cat}$$

(2) Lineweaver-Burk plot

A second useful plot for enzyme kinetics is called the Lineweaver-Burk plot. It is useful because it is linear in 1/[S].

$$\frac{1}{v} = \frac{[S]+K_m}{K_{cat}[E]_0[S]} = \frac{1}{k_{cat}[E]_0} + \frac{K_m}{k_{cat}[E]_0}\cdot\frac{1}{[S]}$$



Notice that with this type of plot, it is possible to obtain K_m and v_{max} without measuring $[E]_0$. However, in order to determine $k_{cat} = v_{max}/[E]_0$, one needs an accurate measurement of $[E]_0$. To find accurate values of the slope and intercept, it is necessary to sample a wide range of values spaced roughly equally in 1/[S]. One usually sets up reactions with a variety of initial substrate concentrations and measures the initial rate for each to obtain the data for this plot.