Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P$$

Turnover is controlled by k_2.

Satisfied by initial rates or velocities and intial $[S] >> E_0$, whence $[P] \rightarrow 0$.

$$\text{Rate} = \text{velocity} = v \cong k_2 [ES] = \frac{k_2 E_0 [S]}{K_M + [S]} = \frac{V_{\max} [S]}{K_M + [S]}$$

where $K_M = \dfrac{k_{-1} + k_2}{k_1}$ $\Leftarrow$ Michaelis constant

and $V_{\max} = k_2 E_0$ k_2 is sometimes called k_{cat}

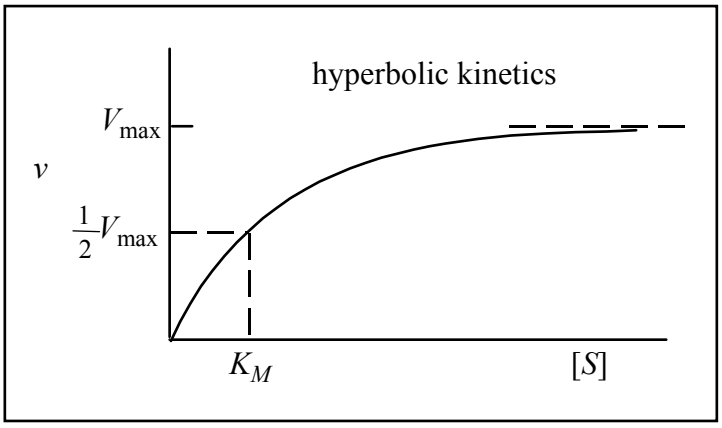


Figure 7-1

Kinetic Plots

$$v = \frac{V_{\max} [S]}{K_M + [S]}$$ hyperbolic

where $v \equiv$ initial rates

$[S] \equiv$ initial substrate concentrations $>> E_0$

Linearized Plots

(1) Lineweaver-Burk: 1/*v* vs 1/[*S*] — Double Reciprocal

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \cdot \frac{1}{[S]}$$

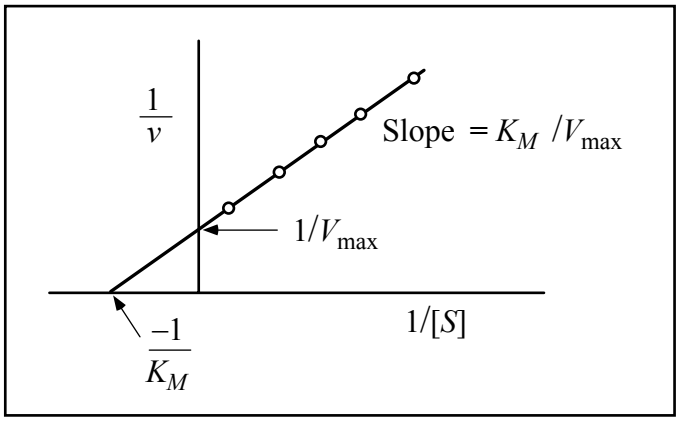


Figure 7-2

(2) Eadie-Hofster: *v*/[*S*] vs *v* — Single Reciprocal

$$\frac{v}{[S]} = \frac{V_{\max} - v}{K_M}$$

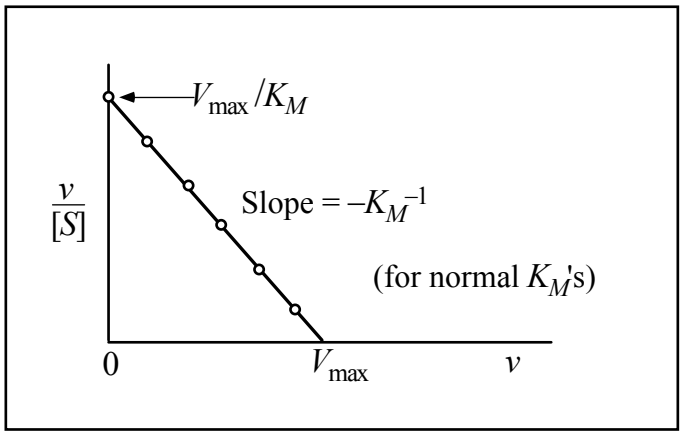


Figure 7-3

(3) Dixon: [*S*]/*v* vs [*S*]

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

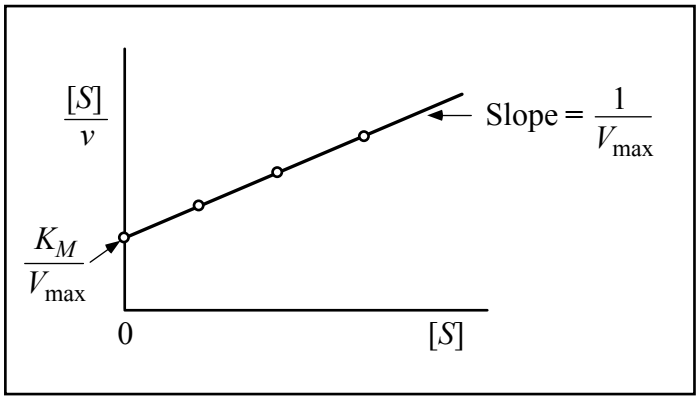


Figure 7-4

Inhibition of Enzymatic Reactions

(1) Competitive Inhibition

A competitive inhibitor is a molecule that resembles the substrate and occupies the catalytic site because of its similarity in structure, but is completely unreactive. By occupying the active site, the inhibitor prevents normal substrates from binding and being catalyzed. Operationally, competitive inhibitors bind reversibly to the active site. Hence, inhibition can be reversed by (1) diluting the inhibitor, or (2) swamping the system with excess substrate.

Reaction Mechanism

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

$$E + I \rightleftarrows EI$$

Define $K_I = \dfrac{[E][I]}{[EI]}$

Expectation

A competitive inhibitor increases K_M, but does not affect V_{max} (because of sufficiently high [*S*], *S* will displace *I*).

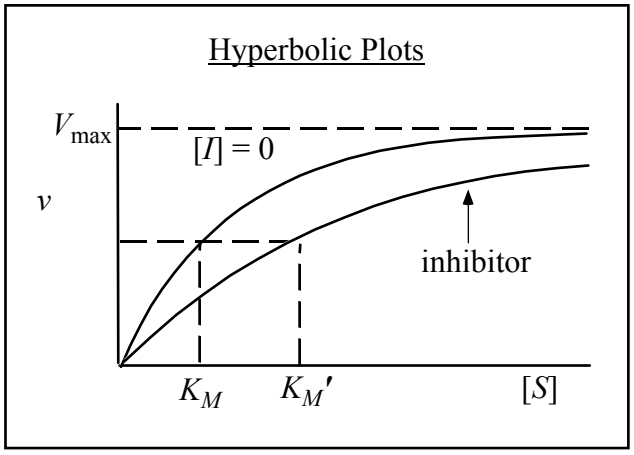


Figure 7-5

$$v = \frac{V_{max}[S]}{[S] + K_M\left[1 + \frac{[I]}{K_I}\right]} = \frac{V_{max}[S]}{[S] + K_M'}$$

where $K_M' = K_M\left[1 + \frac{[I]}{K_I}\right]$ $\Leftarrow$ modified K_M

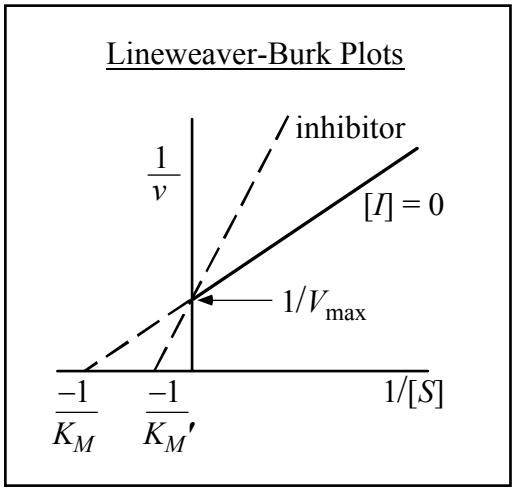


Figure 7-6

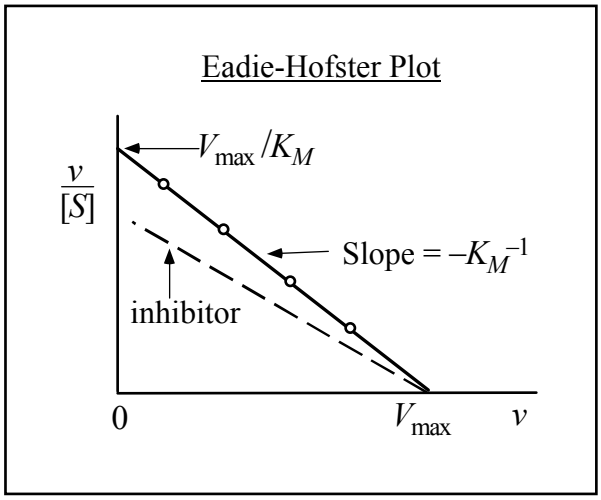


Figure 7-7

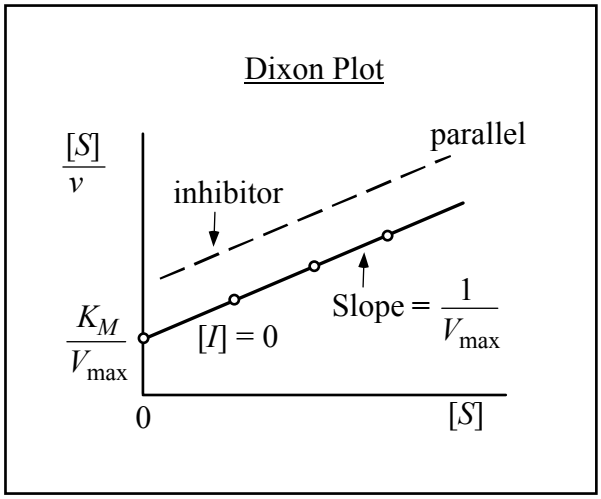
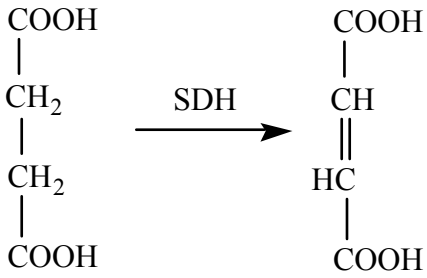


Figure 7-8

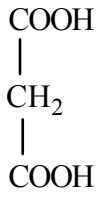
Classical Example of Competitive Inhibition

Enzyme: succinic dehydrogenase (SDH), which catalyzes the oxidation of succinic acid to fumaric acid.



succinic acid fumaric acid

Here substrate ≡ succinic acid
inhibitor ≡ malonic acid



Here $K_I = 1 \times 10^{-5}\,\mathrm{M}$, which means that at $[\text{malmate}] = 10^{-5}\,\mathrm{M}$, the <u>apparent</u> affinity of the enzyme for succinate decreases by a factor of 2!

(2) Noncompetitive Inhibition

A noncompetitive inhibitor is one that binds reversibly to the enzyme, but not at the active site itself, so that the substrate can still bind at the active site, but there's no catalyzed transformation.

This type of inhibition <u>cannot</u> be overcome by a large amount of substrate, thus noncompetitive inhibition.

Reaction Mechanism

$$\begin{array}{ccccc} E + S & \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} & ES & \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} & E + P \\ + & & + & & \\ I & & I & & \\ \downarrow\uparrow & & \downarrow\uparrow & & \\ EI & & ES\cdot I & & \end{array}$$

Define $K_I = \left(\dfrac{[E][I]}{[EI]}\right)_{eq}$ $K_{SI} = \left(\dfrac{[ES][I]}{[ES\cdot I]}\right)_{eq}$

Expectation

The rate or velocity decreases to the extent that E is complexed by inhibitor, irrespective of EI or $ES \cdot I$. Thus, a noncompetitive inhibitor decreases V_{max} without affecting the apparent K_M. The situation is not overcome by swamping the system with substrate.

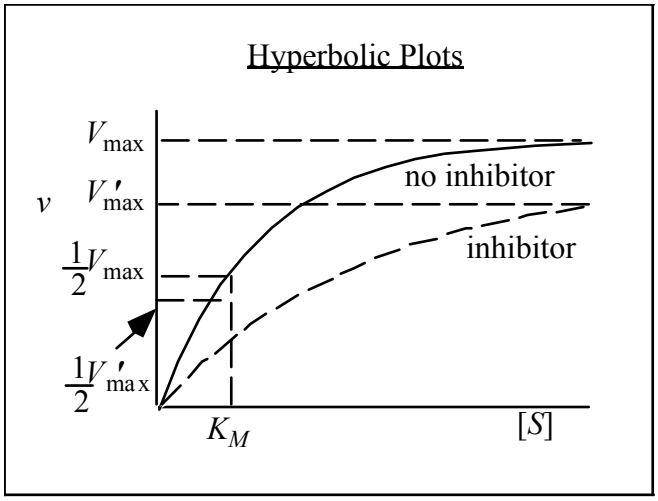


Figure 7-9

Typical Case

$K_I = K_{SI}$, i.e., the affinity of the inhibitor site for the inhibitor does not depend on whether E is bound with S. In this instance

$$v = \frac{V_{max}[S]}{\left(1 + \frac{[I]}{K_I}\right)([S] + K_M)} = \frac{V'_{max}[S]}{K_M + [S]}$$

where $V'_{max} = \frac{1}{\left(1 + \frac{[I]}{K_I}\right)} \cdot V_{max}$

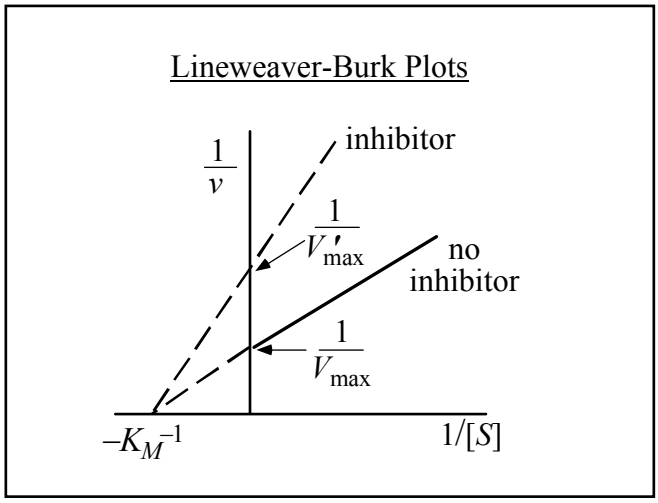


Figure 7-10

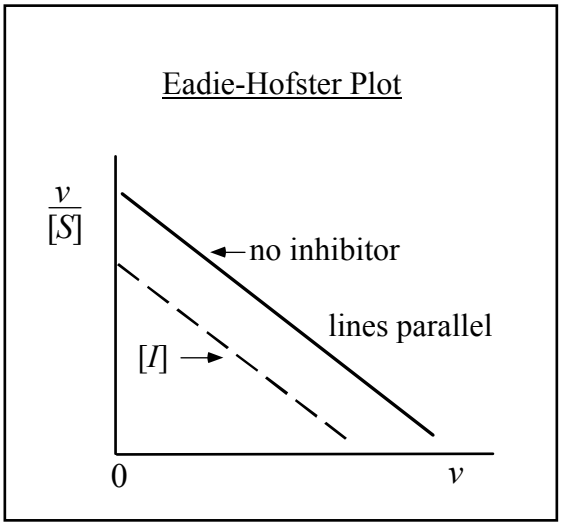


Figure 7-11

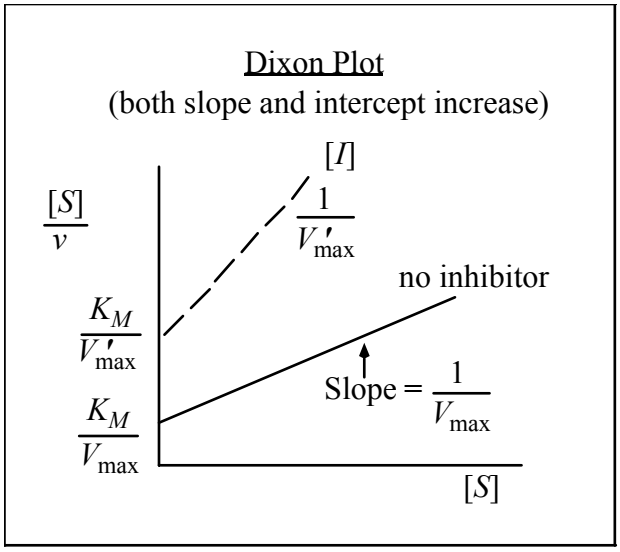


Figure 7-12

More General Case

$K_I \neq K_{SI}$ allosteric interaction between catalytic and inhibitor sites.

$$v = \frac{k_2 E_0 [S]}{\left(1 + \frac{[I]}{K_{SI}}\right)} \cdot \frac{1}{\left(\frac{\alpha}{\beta}\right) K_M + [S]} = \frac{V'_{max} [S]}{\left(\frac{\alpha}{\beta}\right) K_M + [S]}$$

where $\left(\frac{\alpha}{\beta}\right) = \frac{\left(1 + \frac{[I]}{K_I}\right)}{\left(1 + \frac{[I]}{K_{SI}}\right)}$ and $V'_{max} = \frac{V_{max}}{\beta}$

Both V_{max} and K_M can be affected!

Case of $K_{SI} >> K_I$

This is an interesting situation where the allosteric interaction between catalytic and inhibitor sites is so strong that binding is mutually exclusive.

$$v = \frac{1}{\beta} \frac{V_{\max}[S]}{\left(\frac{\alpha}{\beta}\right) K_M + [S]}$$

If $K_{SI} >> [I]$, $\beta \rightarrow 1$

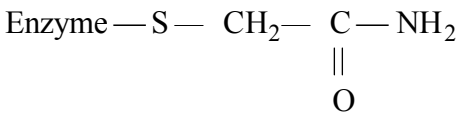
then $$v = \frac{V_{\max}[S]}{\left(1 + \frac{[I]}{K_I}\right) K_M + [S]} = \frac{V_{\max}[S]}{K'_M + [S]}$$

which is the result for competitive inhibition!

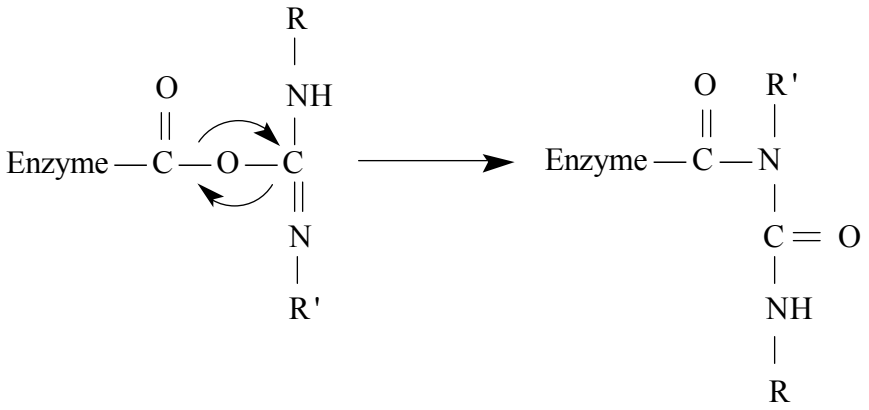
Irreversible Modification

Permanent or irreversible modification of the active site often yields a situation that behaves like the case of simple noncompetitive inhibition!

For example, irreversible modification by the chemical alkylating agent iodoacetamide, which reacts with exposed sulfhydryl groups such as a cysteine to form a covalently modified



or irreversible modification by carbodiimide of carboxyl groups from glutamate and aspartate.



Oftentimes, chemically modified enzyme is inactive catalytically. So, $v \propto [\text{Enzyme}]_{\text{active}}$, i.e., enzyme not chemically modified and unreacted enzyme behave normally with K_M identical to the situation prior to the addition of "inhibitor."

In fact

$$v = \frac{f_{\text{unmodified}}\, V_{\max}\,[S]}{K_M + [S]}$$

like in simple noncompetitive inhibition.

Well-Studied Enzyme System That Behaves According to Michaelis-Menten Kinetics

Ferriprotoporphyrin and $(Mn)_2$ protein catalase; catalyze the decomposition of H_2O_2.

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

Table 7-1. Velocities and Energies for Protein Catalases

Catalyst	Velocity[a], $\frac{-d[H_2O_2]}{dt}$, M S^{-1}	E_a (kJ/mole)
None	10^{-8}	71
HBr	10^{-4}	50
Fe^{2+}/Fe^{3+}	10^{-3}	42
Hematin or Hb	10^{-1}	—
$Fe(OH)_2$ TETA^{+} [b]	10^{3}	29
Catalase	10^{7}	8

[a] for $[H_2O_2] = 1\,M$, $[\text{catalyst}]_{\text{active sites}} = 1\,M$
[b] triethylenetetramine

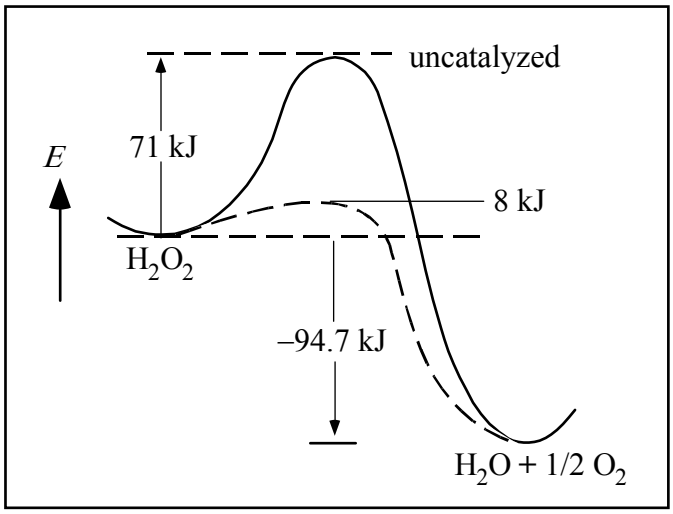


Figure 7-13

$$\Delta G^0_{298} = -103.10 \text{ kJ mol}^{-1} \quad \text{for} \quad H_2O_2(aq) \rightarrow H_2O\,(l) + \frac{1}{2}O_2\,(g)$$

$$\Delta H^0_{298} = -94.64 \text{ kJ mol}^{-1}$$

Turnover number (S^{-1}) ≡ maximum velocity divided by the concentration of enzyme active sites

Table 7-2. Turnover Numbers for Various Enzymes and Substrates

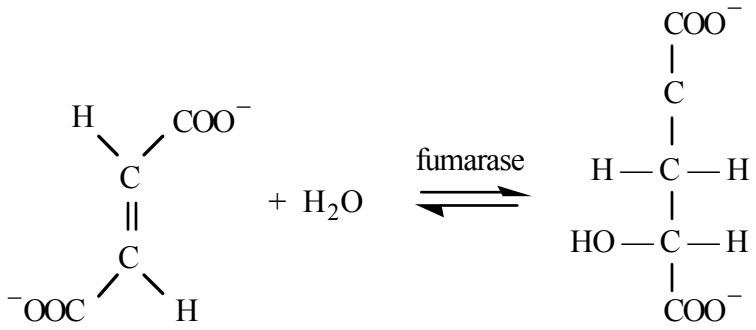
Enzyme	Substrate	Turnover No. (S^{-1})*
Catalase	H_2O_2	9×10^6
Acetylcholinesterase	Acetylcholine	1.2×10^4
Lactate dehydrogenase (chicken)	Pyruvate	6×10^3
Chymotrypsin	Acetyl-L-tyrosine ethyl ester	4.3×10^2
Myosin	ATP	3
Fumarase	L-Malate	1.1×10^3
	Fumarate	2.5×10^3
Carbonic anhydrase (bovine)	CO_2	8×10^4
	HCO_3^-	3×10^4

*Typically, turnover number ~ 10^3 S^{-1} within a factor of 10.

Two Intermediate Complexes

The reaction catalyzed by catalase is essentially irreversible. Therefore, it is not necessary to worry about *EP*. Most enzymatic reactions are readily reversible, so an enzyme-product complex can often be detected.

Good Example



fumarate L-malate

Equilibrium	20%	80%	at room temperature

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P$$

So in this case, you cannot ignore *EP*! More complete treatment leads to the following results:

(1) Forward Reaction: $[P]_0 = 0$

$$v_F = \left(\frac{d[P]}{dt}\right)_0 = k_3[EP]$$

$$= \frac{k_2 k_3 E_0 [S]}{\left(k_2 + k_{-2} + k_3\right)\left\{\dfrac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_1\left(k_2 + k_{-2} + k_3\right)} + [S]\right\}}$$

which can be reduced to

$$v_F = \frac{V_F[S]}{K_M^F + [S]}$$

with $V_F = \dfrac{k_2 k_3 E_0}{k_2 + k_{-2} + k_3}$ and $K_M^F = \dfrac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_1\left(k_2 + k_{-2} + k_3\right)}$

(2) Reverse Reaction: $[S]_0 = 0$[1]

$$v_R = \left(\frac{d[S]}{dt}\right)_0 = k_{-1}[ES]$$

$$= \frac{V_R[P]}{K_M^R + [P]}$$

with $V_R = \dfrac{k_{-1}k_{-2}E_0}{k_{-1} + k_2 + k_{-2}}$ and $K_M^R = \dfrac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_{-3}\left(k_{-1} + k_2 + k_{-2}\right)}$

(3) Net Velocity

$$v = \frac{V_F K_M^R[S] - V_R K_M^F[P]}{K_M^F K_M^R + K_M^R[S] + K_M^F[P]}$$

Note that at equilibrium, $v = 0$ and hence

$$V_F K_M^R[S]_{eq} = V_R K_M^F[P]_{eq} \quad \text{and}$$

$$K = \left(\frac{[P]}{[S]}\right)_{eq} = \frac{V_F K_M^R}{V_R K_M^F}$$

There are too many kinetic constants here to be sorted out by steady state kinetics.

We must appeal to the methods of rapid kinetics.

[1]Alberty and Peirce, *J. Am. Chem. Soc.* **79**, 1526 (1957).