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Fundamentals of Biochemistry

Second Edition

Chapter 12:

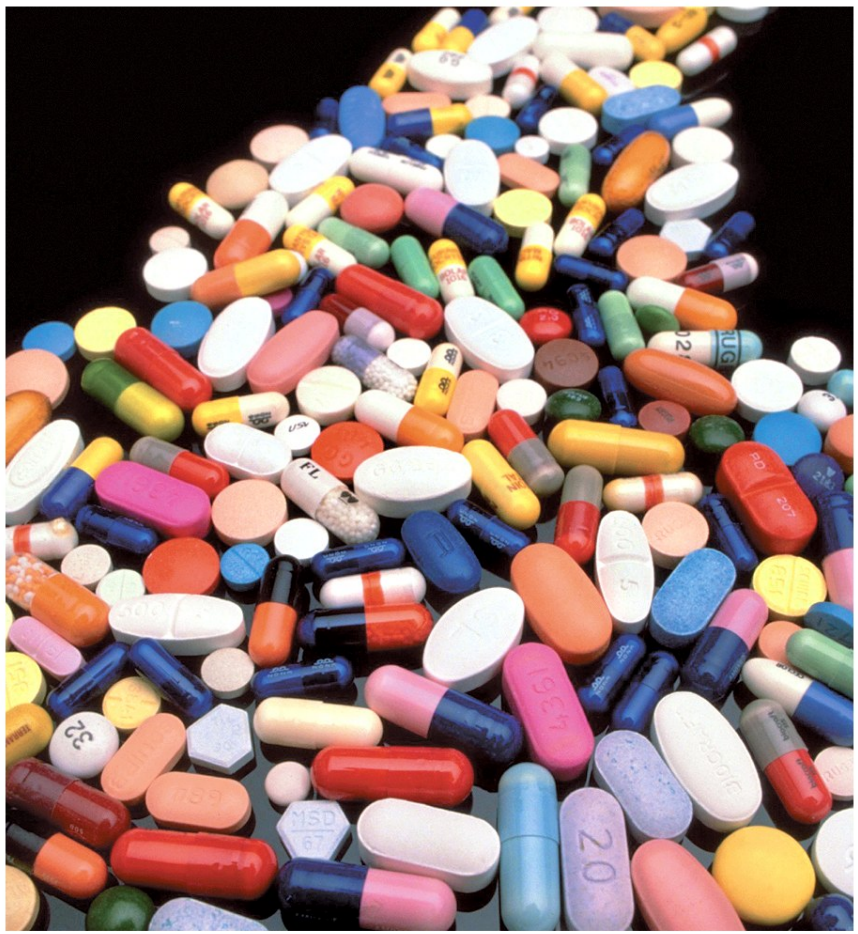
Enzyme Kinetics, Inhibition, and Regulation

Enzyme kinetics

The study of enzymatic reaction rates

Enzyme structure and catalytic mechanism

→ Biological function
Disease therapy



Chapter 12 Opener Fundamentals of Biochemistry, 2/e

Reaction kinetics

A. Chemical kinetics

$A \rightarrow P$: overall reaction process

$A \rightarrow I_1 \rightarrow I_2 \rightarrow P$: elementary reactions

Reaction order

$v = d[P]/dt = - d[A]/dt = k[A]$

Reaction rate is proportional to the frequency with which the reacting molecules come together

Reaction rate is measured by the disappearance of reactant
the appearance of product

k: rate constant

v: reaction velocity

First-order

Proportional to the conc. of the substance A: unimolecular reaction

Velocity unit: Ms^{-1}

K: s^{-1}

Second-order

$$2A \rightarrow P$$

$$A + B \rightarrow P$$

$v = -d[A]/dt = - d[B]/dt = k[A][B]$

k unit = $M^{-1}\ s^{-1}$

Rate equations

the progress of a reaction as a function of time

$v = d[P]/dt = - d[A]/dt = k[A]$

$d[A]/[A] = d \ln [A] = - k \, dt$

Integration

$\ln [A] = \ln [A]_o - kt$

A plot of a first-order rate equation

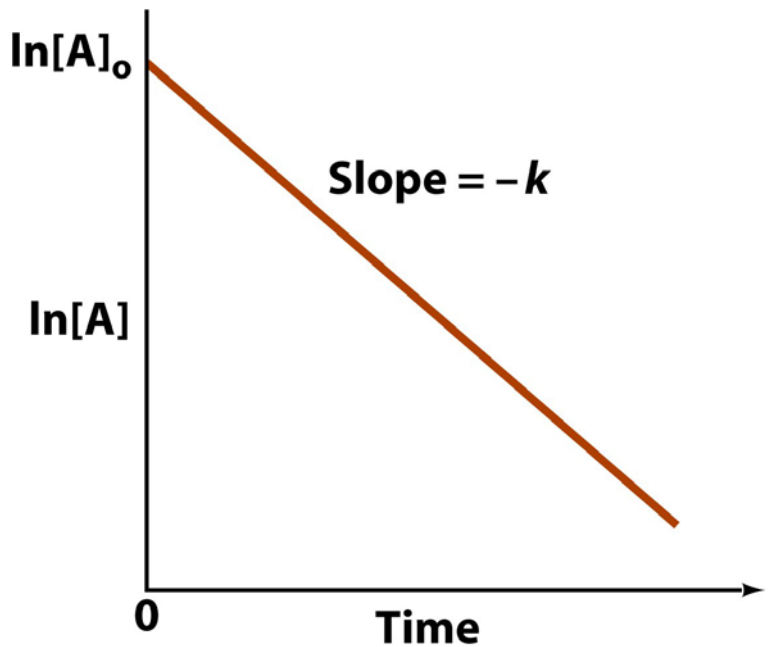


Figure 12-1 Fundamentals of Biochemistry, 2/e

$t_{1/2}$ = Half-life (half-time)

$\ln [A] = \ln [A]_o - kt$
If $[A] = [A]_o /2$
$\ln ([A]_o /2 / [A]_o) = - k\, t_{1/2}$
$t_{½} = \ln 2/k = 0.693/k$

Radioactive decay

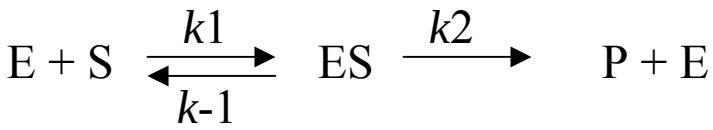
Radionuclide	Half-life	Type of Radiation[a]
^{3}H	12 years	β
^{14}C	5715 years	β
^{24}Na	15 hours	β
^{32}P	14 days	β
^{35}S	87 days	β
^{40}K	1.25×10^{9} years	β
^{45}Ca	163 days	β
^{125}I	59 days	γ
^{131}I	8 days	β, γ

[a] β particles are emitted electrons, and γ rays are emitted photons.

Box 12-1 Table 1 Fundamentals of Biochemistry, 2/e

B. Enzyme kinetics

Enzyme substrates: from a single to more



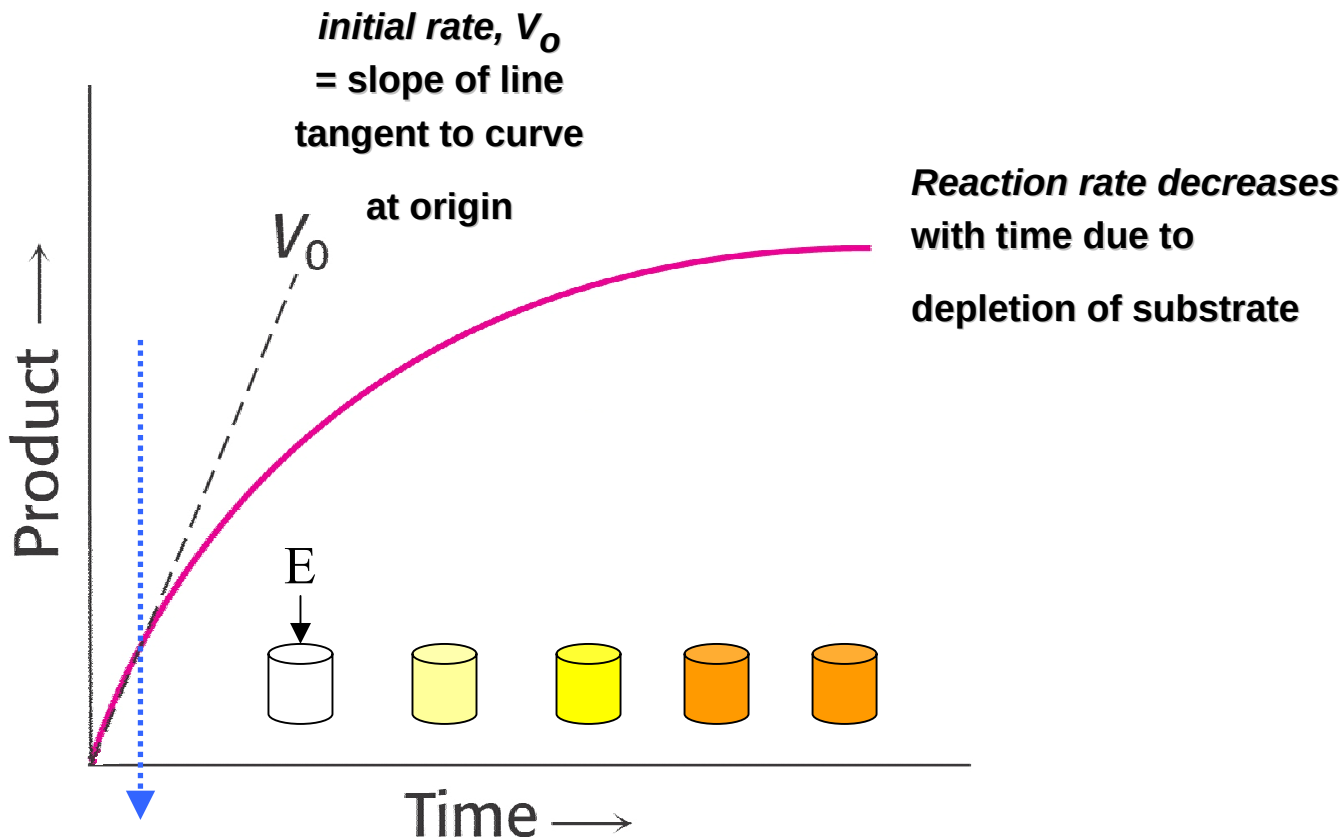
ES: enzyme-substrate complex
Second reaction is irreversible: no P is converted back to S

-Michaelis-Menten equation and

-the definitions of V_{max} and K_m for an enzyme-catalyzed reaction

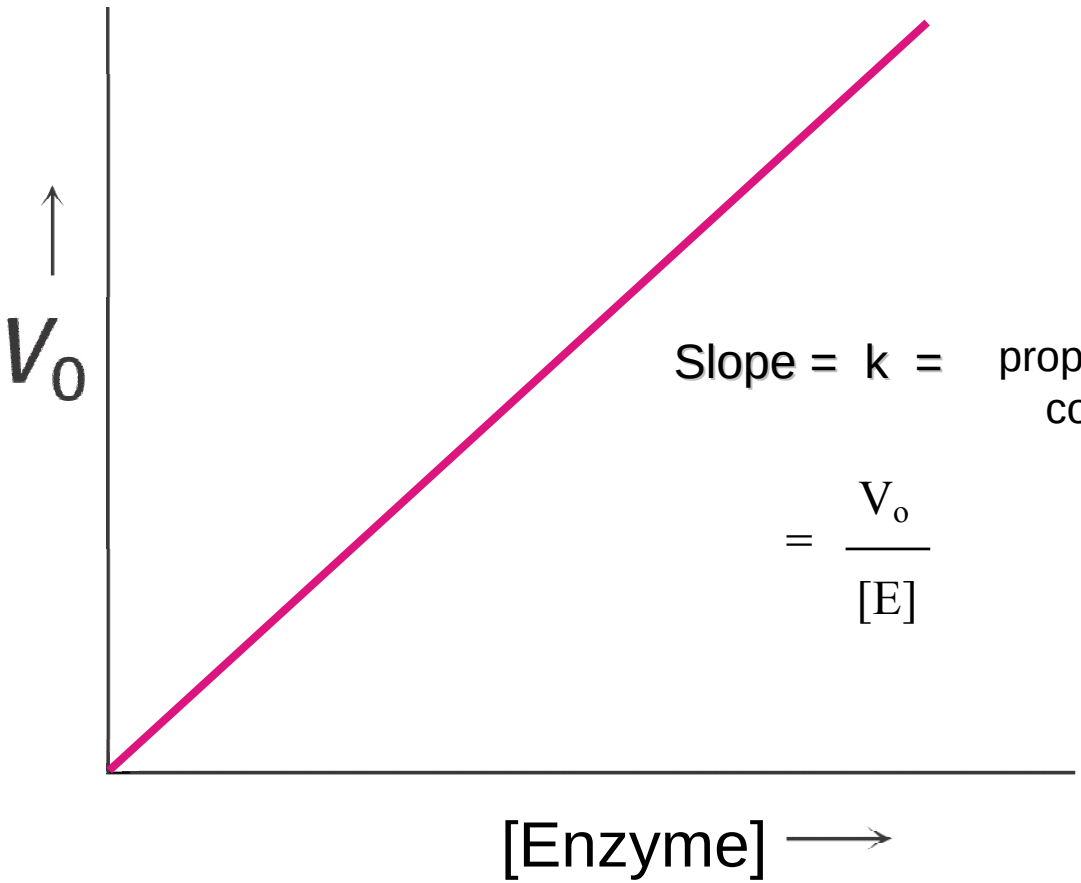
Enzyme reaction: dependent on time, enzyme, substrate

Function of time- enzyme, substrate constant



Rates are estimated by V_o at limiting substrate concentrations

Function of enzyme concentration-time, substrate constant



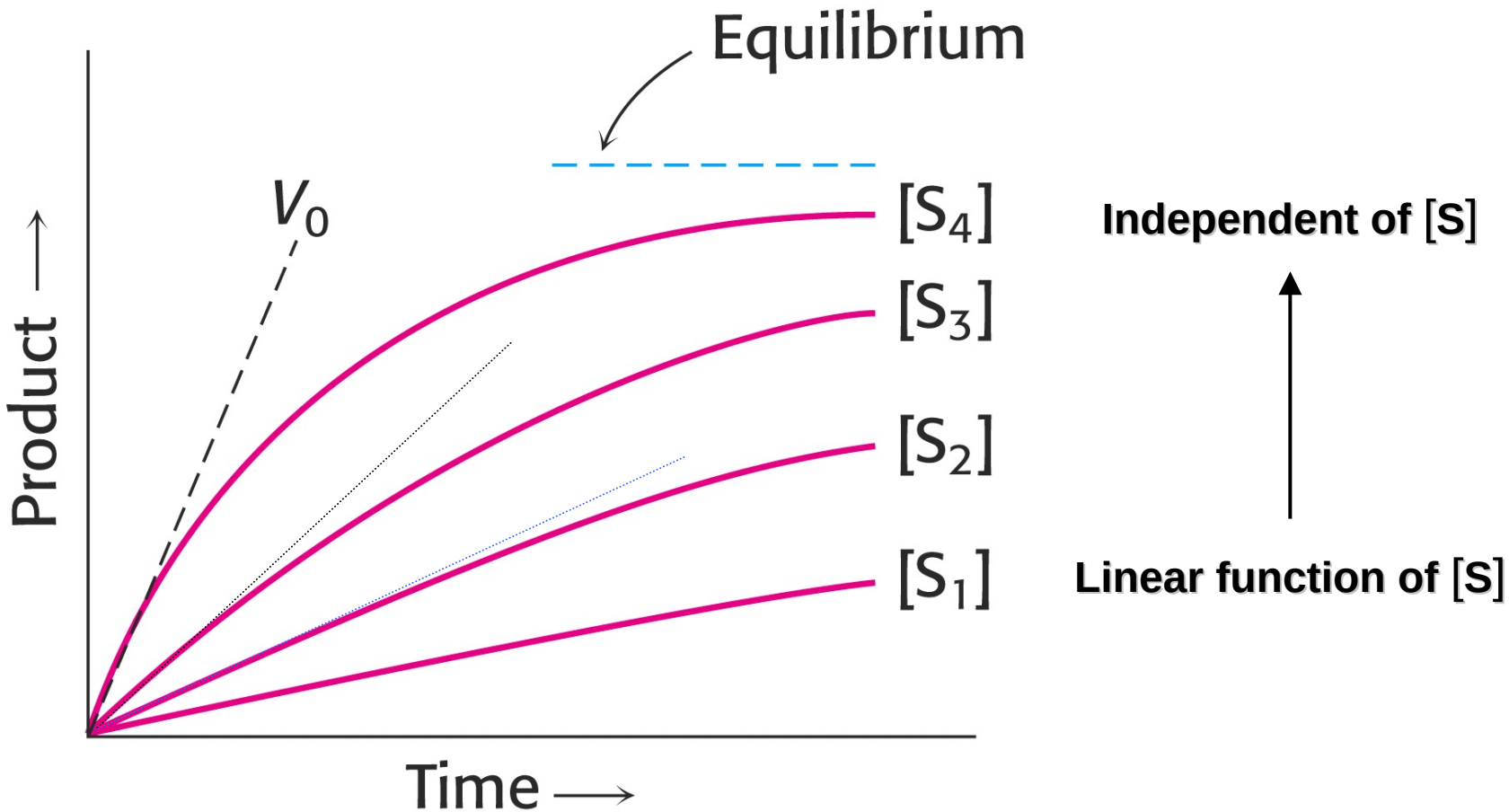
Slope = k = a proportionality constant

$$= \frac{V_o}{[E]}$$

$$V_o = k\,[E]$$

Initial velocities increase *linearly* as a function of enzyme concentration.

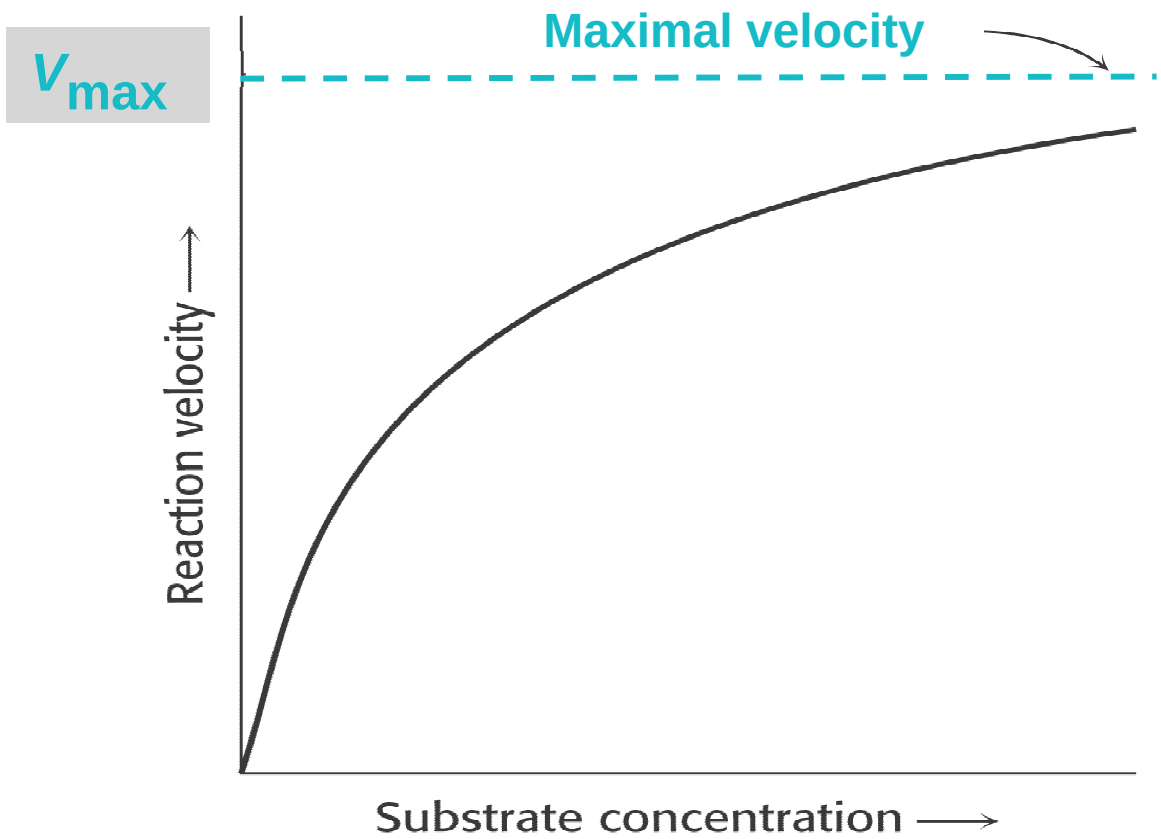
Effect of substrate: rates over a range of substrate concentrations



At low concentrations, V_o is a first-order function of [S],

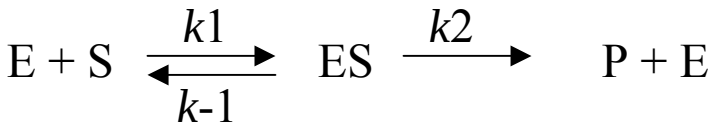
whereas at high concentrations V_o is independent of [S]

Function of substrate concentration



At high concentrations, V_o becomes independent of [S], reaching its maximal value, V_{max}

Michaelis-Menten equation



V_o becomes independent of [S] at high concentrations

because the enzyme active sites become saturated,

limiting the reaction

to a steady-state concentration of enzyme-substrate complex, [ES].

Initial velocity equals rate of product formation, so:

$$V_o = \frac{d[P]}{dt} = k_2[ES]$$

assumptions

approximations

Michaelis-Menten equation

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

Michaelis constant, K_M

The progress curves for a simple enzyme-catalyzed reaction

For [ES] to remain constant, the rate of formation of ES must equal the rate of breakdown of ES.

rate of formation = rate of breakdown

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

$$k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

steady state assumption

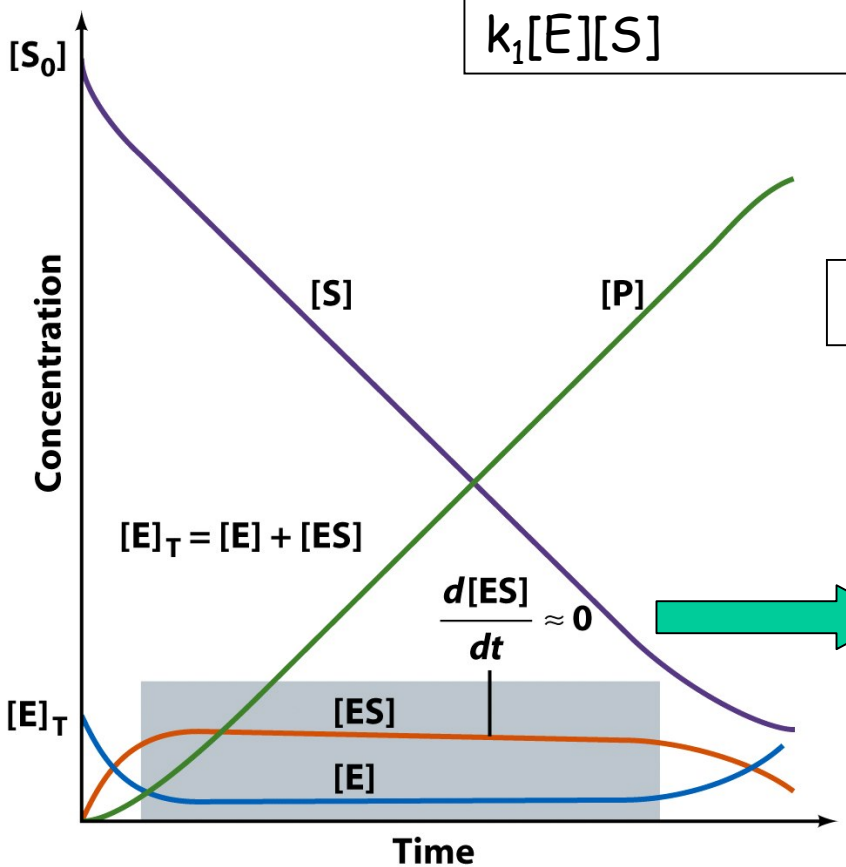
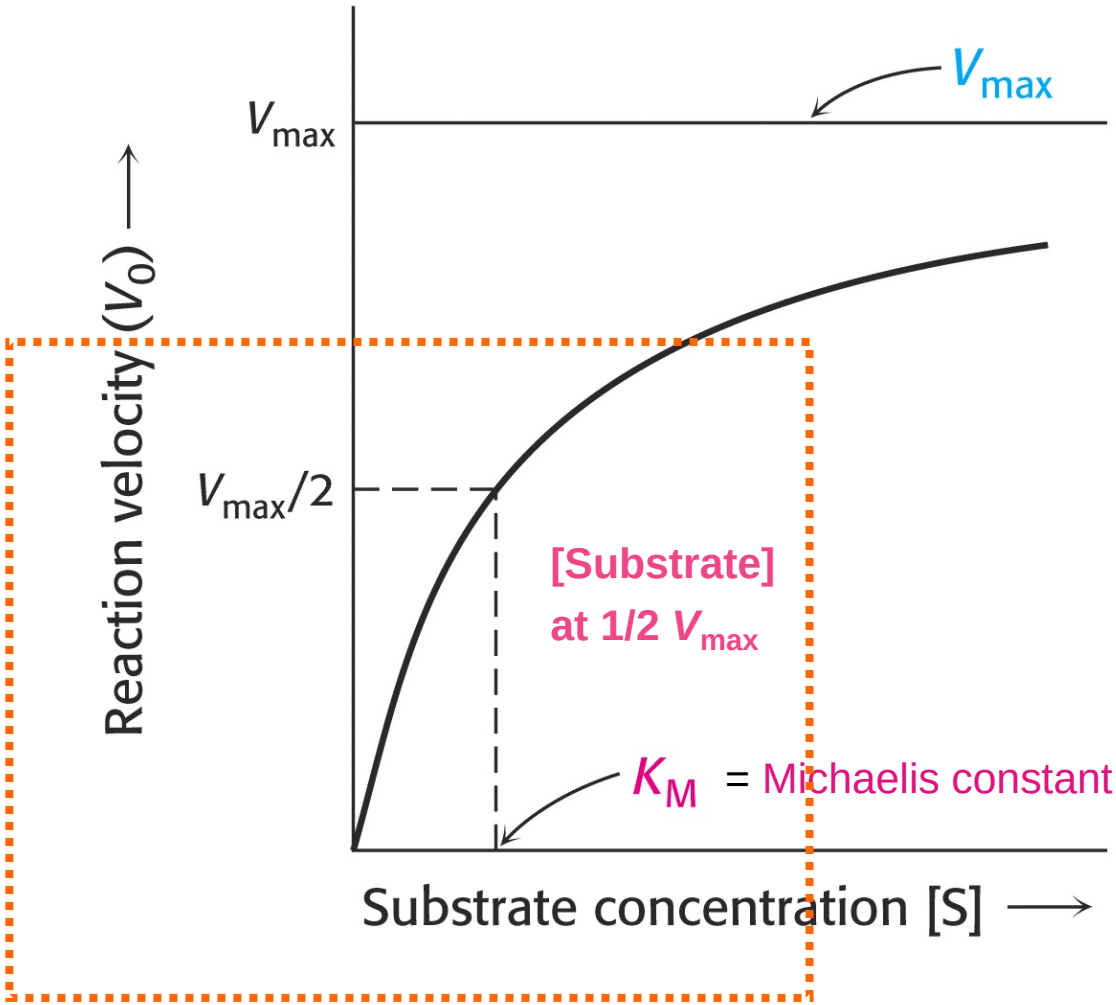


Figure 12-2 Fundamentals of Biochemistry, 2/e

Michaelis-Menten parameters- hyperbolic substrate saturation plot



function of [enzyme]

characteristic parameter of an enzyme

V_o approaches V_{max} *asymptotically* at saturating concentrations

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

1. $[S] << K_m$ $\quad V_o = \dfrac{V_{max}[S]}{K_m}$

2. $[S] >> K_m$ $\quad V_o = \dfrac{V_{max}[S]}{[S]}$

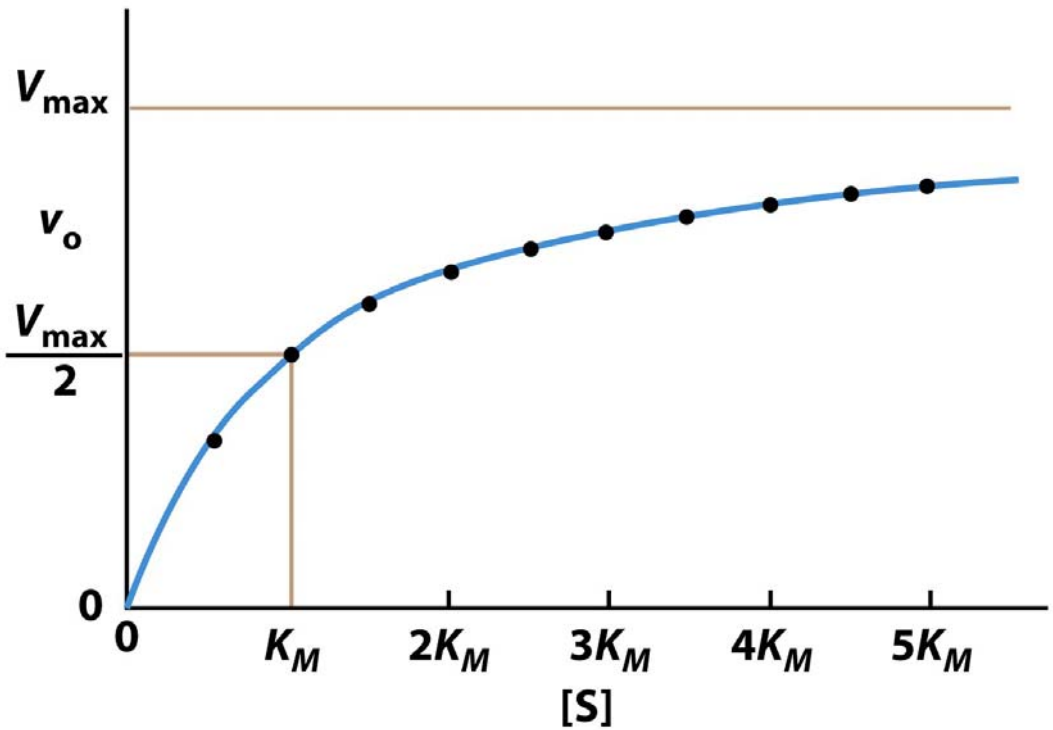


Figure 12-3 Fundamentals of Biochemistry, 2/e

$[S] = 1\ K_M$
$= 2\ K_M$
$= 3\ K_M$
$= 4\ K_M$
$= 5\ K_M$

$V_o = ?$

Table 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Accetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Table 12-1 Fundamentals of Biochemistry, 2/e

Catalytic efficiency (k_{cat}/K_M)

The turnover number $k_{cat} = V_{max}/[E_T]$
($k_{cat} = k_2$ in simple system).
k_{cat} is the limiting rate at enzyme saturation
How may substrate per enzyme (or active site) per second

When $[S] << K_M$
very little ES is formed and $[E] = [E]_T$

$$V_o = \frac{V_{max}[S]}{K_M + [S]} \longrightarrow V_o = \frac{k_{cat}[E_T][S]}{K_M + [S]} \longrightarrow V_o = \frac{k_{cat}}{K_M}[E_T][S]$$

k_{cat}/K_M is a second-order constant
Indicating how often enzyme and substrate encounter one another in solution
Therefore a measure of an enzyme's catalytic efficiency
There is an upper limit: can't be greater than k_1 (what does it mean?)
Catalytic perfection: $k_{cat}/K_M = 10^8 \sim 10^9\ M^{-1}\ s^{-1}$

Rearranged Michaelis-Menten equation- parameters from a straight line

Lineweaver-Burk equation

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

Unnumbered figure pg 367 Fundamentals of Biochemistry, 2/e

A double-reciprocal (Lineweaver-Burk) plot

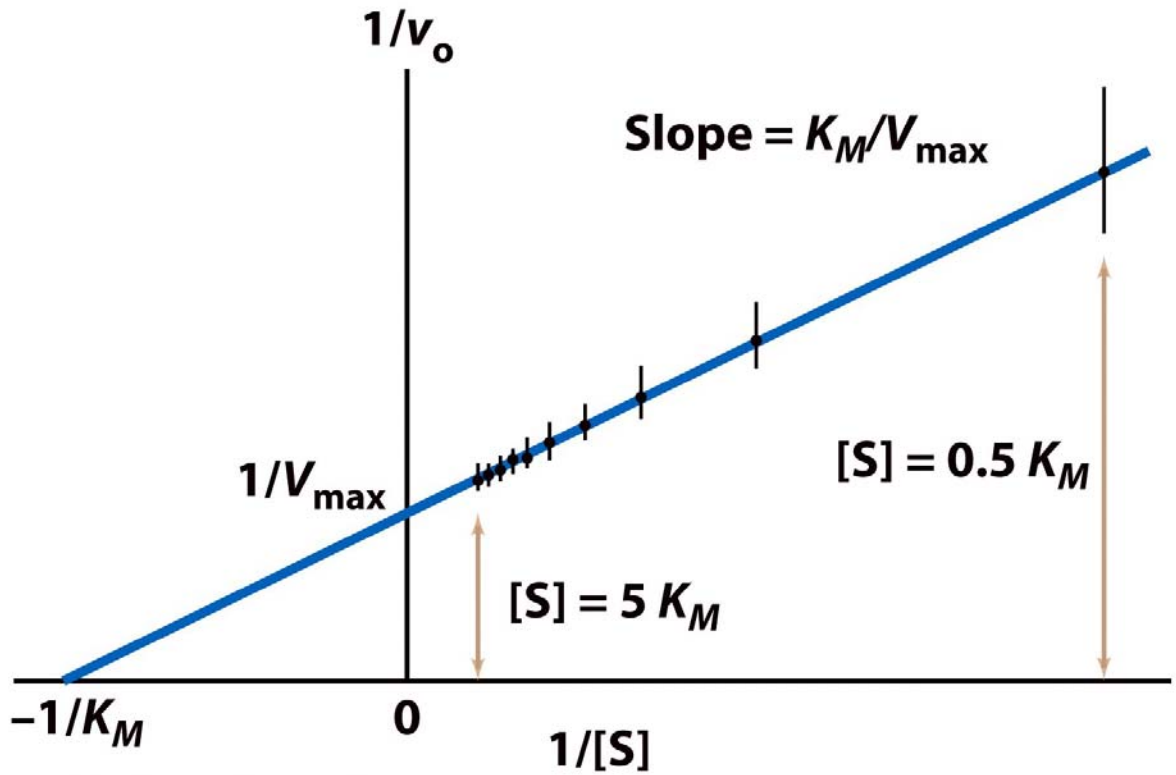
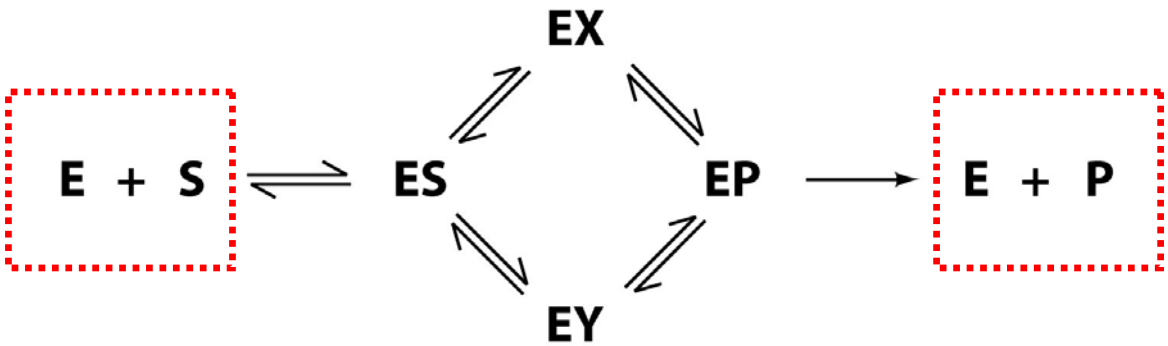


Figure 12-4 Fundamentals of Biochemistry, 2/e

Values for K_m and V_{max} can be determined from a plot of $1/V_o$ vs 1/[S]

Steady state kinetics cannot unambiguously establish a reaction mechanism



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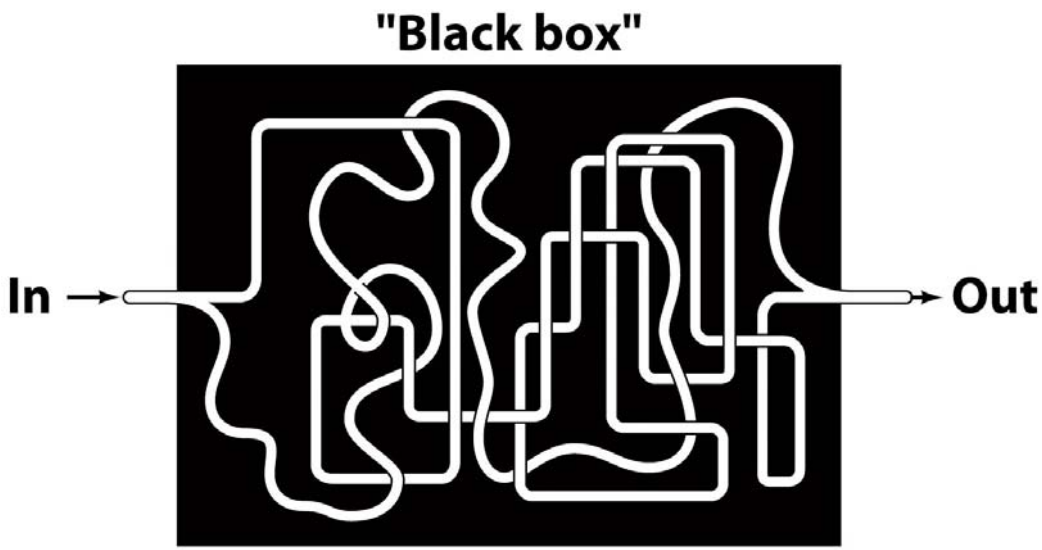

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Bisubstrate reactions

two substrates: ~60% of known biochemical reactions

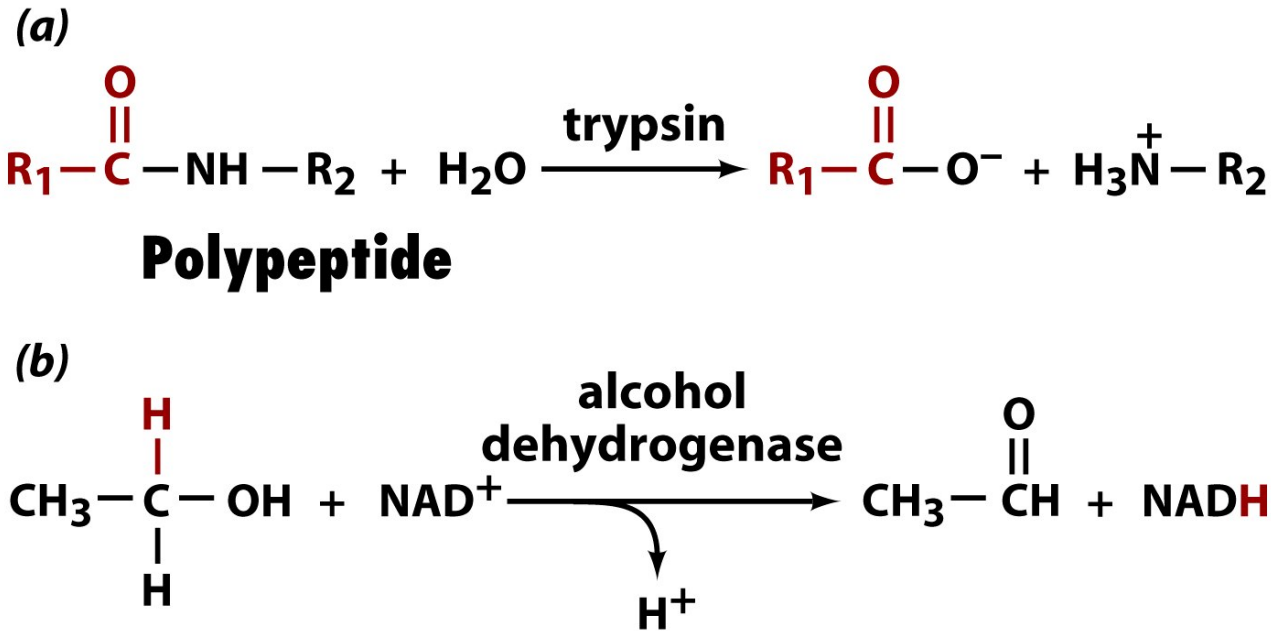

Figure 12-5 Fundamentals of Biochemistry, 2/e
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Sequential reactions: single-displacement reactions

Ordered mechanism

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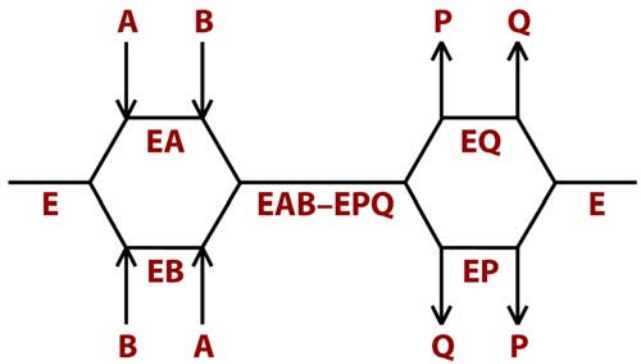
Random mechanism



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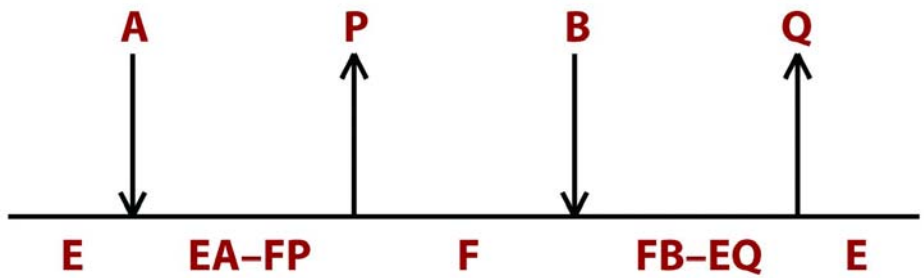
Ping pong reactions: double-displacement reactions



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