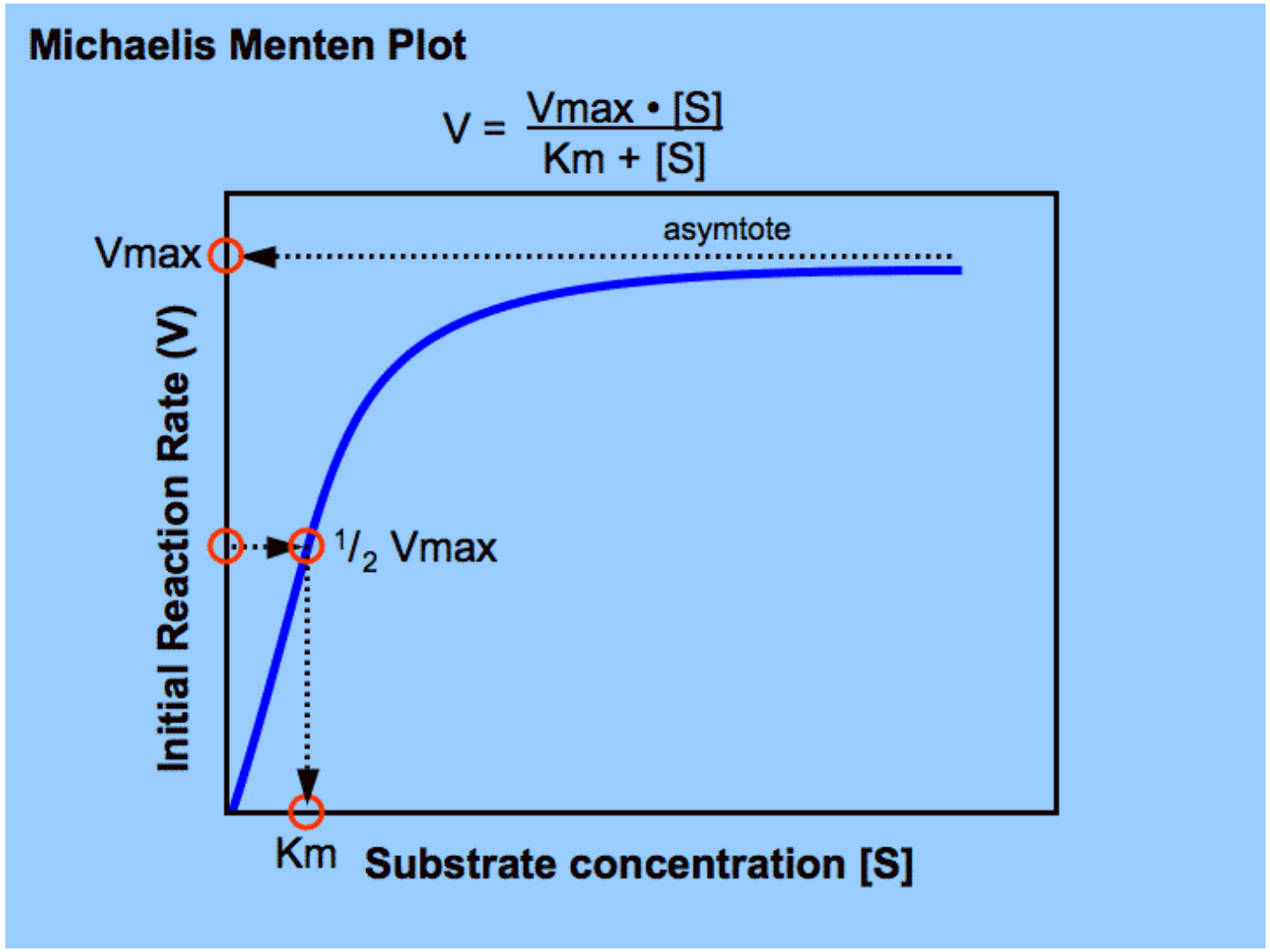
Lecture #3: **Michaelis-Menten Kinetics**

(1) Introduction
(2) Assumptions
(3) Derivation
(4) Description of v_o versus [S] curves
(5) Michaelis constant (K_M)
(6) Specificity/Substrate constant (SpC)
(7) Appendix



Michaelis-Menten Kinetics


Leonor Michaelis
1875–1949


Maud Menten
1879–1960

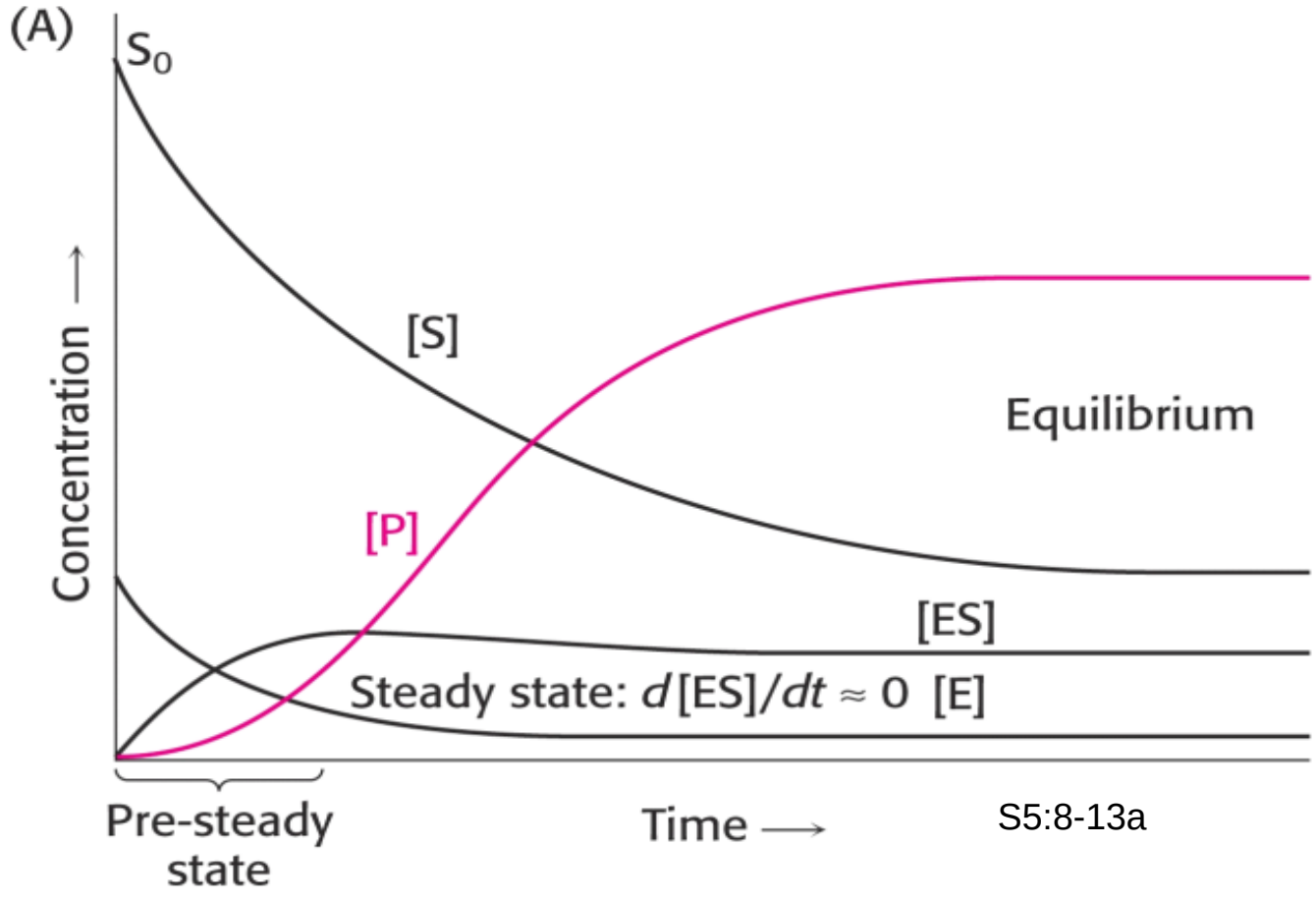
(1) Introduction

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

-The M-M treatment (rapid equilibrium approximation ≡ 1913) is unsuitable for many biochemical systems because:

(a) Many intermediates in enzyme reactions are reactive intermediates
(b) True equilibrium likely doesn't exist
(c) Biochemical systems are often a chain of reactions

-apply the steady-state treatment to assume that **the rate of ES formation is equal to the rate of ES consumption** over the short time interval defined by the initial velocity



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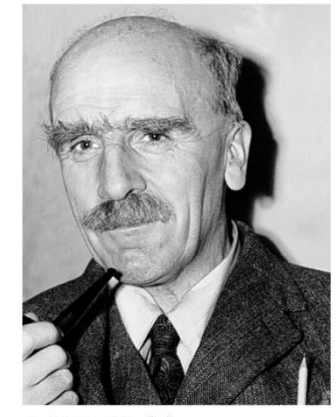
-in **1925 Briggs and Haldane** developed the steady-state treatment

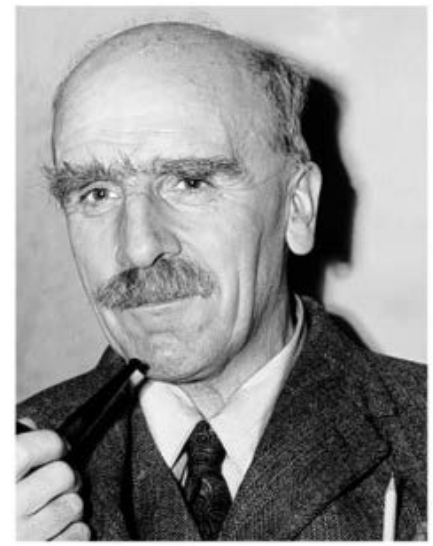

J. B. S. Haldane
1892–1964

2. Assumptions:

(a) the E-S complex is in a **steady-state**

-the rate at which the complex is being formed from E_f and S is equal to the rate at which it is consumed by being broken down to E + P or E + S

$$d[ES]/dt = 0$$



J. B. S. Haldane
1892–1964

(b) The formation of P $\propto$ the concn of ES complex

-the reverse reaction can be ignored (k_{-2} rate)

-implies that the **breakdown of ES to E + P** is *RDS* of reaction

$$v_o = k_2[ES]$$

-implies that reverse reaction (E + P $\rightarrow$ EP $\rightarrow$ ES) is negligible (true if use **initial velocity!)**

(c) The S concn is much larger than E_T concn

-change in [S] as a result of the formation of ES complex is negligible

-the substrate concn is taken to be a constant

-assumptions (b) and (c) are identical to those of M-M treatment

- **Deviation from M-M kinetics happens when:**
  - There are multiple catalytic steps ($k_2 \neq k_{cat}$)
  - [ES] is not at steady state
  - The enzyme displays cooperativity
  - There are multiple substrates
  - Substrate binding is irreversible

3. Derivation

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

-assumption (a): $d[ES]/dt = 0$ {K-1}

Rate of formation of ES = Rate of consumption of ES

Rate of formation = $k_1[E_f][S] + k_{-2}[E_f][P]$ {K-2}

-based on assumption (b) the second term can be ignored (reverse reaction) so:

Rate of ES formation = $k_1[E_f][S]$ {K-3}

Rate of ES consumption = $k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES]$ {K-4}

$$d[ES]/dt = k_1[E_f][S] - (k_{-1} + k_2)[ES] = 0 \quad \{K\text{-}5\}$$

$$k_1[E_f][S] = (k_{-1} + k_2)[ES] \quad \{K\text{-}6\}$$

Mass Conservation Equation for $[E_T]$ at constant [S]

$$[E_T] = [E_f] + [ES] \quad \{K\text{-}7\}$$

and $$[E_f] = [E_T] - [ES] \quad \{K\text{-}8\}$$

-substitute $[E_T] – [ES]$ from {K-8} into {K-6} for $[E_f]$

$k_1([E_T] – [ES])[S] = (k_{-1} + k_2)[ES]$ {K-9} group rate constants

$([E_T] – [ES])[S] = \{(k_{-1} + k_2)/k_1\}[ES]$ {K-10}

-define **a new constant, K_M,** such that: $K_M = (k_{-1} + k_2)/k_1$ {K-11}

-called the **"Michaelis constant"**

-substituting K_M into {K-10} yields:

$([E_T] -[ES])[S] = K_M[ES]$ or

$[E_T][S] - [ES][S] = K_M[ES]$ {K-12} get [ES] out of eq.

-velocity of reaction based on assumption (b) and so:

$v_o = k_2[ES]$ {K-13}

or

$[ES] = v_o/k_2$

-substituting for [ES] into {K-12} gives the rate equation:

$[E_T][S] - v_o/k_2 * [S] = K_M * v_o/k_2$ {K-14}

-rearranging {K-14} gives: (multiply by k_2)

$k_2[E_T][S] - v_o[S] = K_M v_o$ {K-15}

At **saturation** conditions: $k_2 = k_{cat}$

-recall: TN (k_{cat}) = $V_{max}/[E_T]$ so:

$k_2 = V_{max}/[E_T]$ {K-16}

$V_{max} = k_2[E_T]$ {K-17}

-substitute V_{max} into {K-15}

$k_2[E_T][S] - v_o[S] = K_M v_o$ {K-15}

$V_{max}[S] - v_o[S] = K_M v_o$ and rearrange

$V_{max}[S] = K_M v_o + v_o[S]$ group terms

$V_{max}[S] = v_o(K_M + [S])$ solve for v_o

$$v_o = V_{max}[S]/(K_M + [S]) \quad \{K\text{-}18\}$$

Michaelis-Menten Equation!

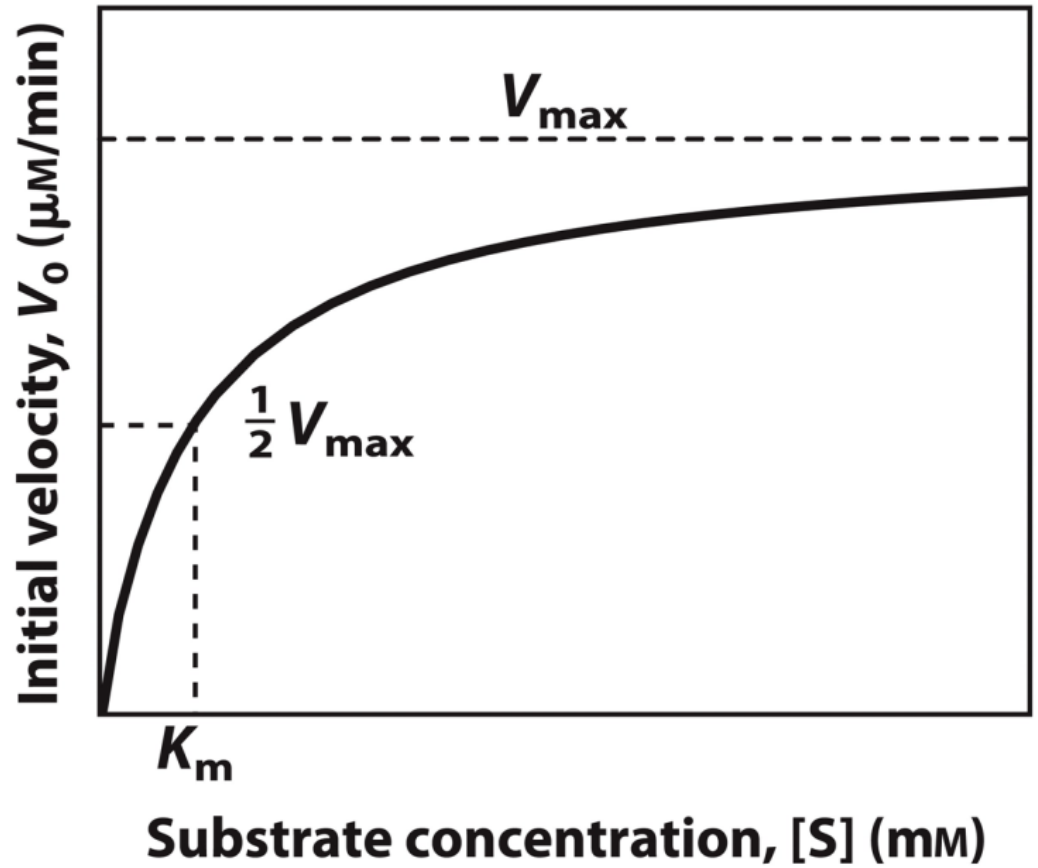


Figure 6-11
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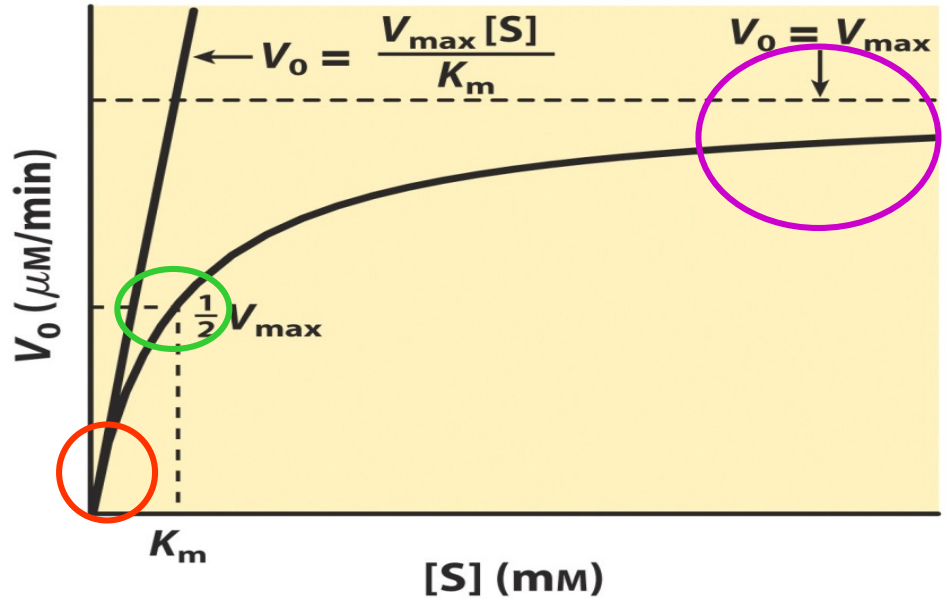

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4. Description of v_o versus [S] curve:

i. When **[S] << K_M** then {K-18} <u>becomes</u>:

$v_o = V_{max}[S]/(K_M + [S]) = V_{max}[S]/K_M = k''[S]$ {K-19}

where $k'' = V_{max}/K_M$ = constant

-{K-19} is rate equation for <u>first order reaction</u> with **v_o α [S]**

-low concn range of v versus [S] curve

ii. When **[S] >> K_M** then {K-18} becomes: (drop K_M)

$v_o = V_{max}[S]/(K_M + [S]) = V_{max}[S]/[S] = V_{max}$ {K-20}

-{K-20} is rate equation for a **zero order reaction** with v_o independent of [S]

-high end of v_o versus [S] curve

iii. Central part of v_o versus [S] curve is described by intermediate range of [S]

-**special case:** when [S] = K_M

$v_o = V_{max}[S]/(K_M + [S]) = V_{max}[S]/([S] + [S]) = V_{max}/2$

-K_M defined as being **equal to that substrate concn which gives one half of the maximum velocity of reaction**

-K_M has units of concn (M)

5. Michaelis Constant (K_M)

(a) K_M is [S] at which one half of V_{max} of a reaction is observed

(b) K_M has units of concn

(c) K_M is a constant only under rigorously defined conditions of pH, T, ionic strength, etc

-if reaction has 2 or more substrates, the true K_M for a given substrate is when all other [S] are at saturation

-V_{max} is that observed when all substrates are at sat'n concn

(d) Values for K_M vary greatly from E to E

-generally range 10^{-8} to 1.0 M

-large K_M: **means that one half of V_{max} is achieved at a relatively large S concn**

-small K_M: **means that 1/2 of V_{max} occurs at relatively low S concn**

(e) K_M is **not** an equilibrium constant, but rather a complex constant, composed of various rate constants

$K_M = (k_{-1} + k_2)/k_1$ for simple reaction

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

(f) K_M is **not** a dissociation constant of the ES complex

Numerical values of K_M

(a) K_M relates reaction velocity to [S] and indicates the **approximate level of the intracellular [S]**

-unlikely that intracellular [S] would be significantly higher or lower than the K_M

(b) K_M is a **characteristic constant** for a given E under defined conditions

-useful to help identify an enzyme

(c) K_M is useful for comparing the activity of different E and for comparing the "suitability" of alternate substrates for the same E

-a substrate with a lower K_M is a better substrate

(d) best S of an E is one which leads to the **highest V_{max} and the lowest K_M**

-a substrate which yields the highest **V_{max}/K_M ratio**

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Table 6-6
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(e) Knowing the K_M value permits adjustment of assay conditions, one can vary [S] according to the needs of assay

-can then measure total [E] or [S]

(f) K_M values useful in evaluating the relative roles of forward and reverse reactions in metabolism

6. Specificity Constant (SpC)

- recall: (TN or k_{cat}) is the maximum number of moles of substrate that are converted to product each second per mole of enzyme

  -k_{cat} is a measure of **how rapidly an enzyme can operate once the active site is filled**

  •$k_{cat} = V_{max}/[E]$

-under physiological conditions, enzymes **do not operate** at saturating S conditions

-usually the ratio of [S]:K_M is in the range of 0.01 to 1.0.

-previously determined that at low [S] then:

$$v_o = V_{max}[S]/K_M$$

- the number of moles of S converted to P per second per mole of enzyme = $(V_{max}[S]/K_M)/[E] = (k_{cat}/K_M)[S]$

-the ratio k_{cat}/K_M is therefore a measure of **how rapidly an enzyme can work at low [S]**

-known as the **"specificity constant" (SpC)**

-k_{cat}/K_M compares **the relative abilities of different compounds to serve as a substrate for a given enzyme**

- SpC: compares the rate of an enzyme-catalyzed reaction with the rate of random diffusion of the enzyme and substrate

-if every collision between a protein and a small molecule resulted in a reaction, the max possible value of the second-order rate constant would be 10^8 to 10^9 $M^{-1}s^{-1}$

TABLE 8.7 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M (s^{-1} M^{-1})
Glycine	—H	1.3×10^{-1}
Valine	—CH(CH$_3$)$_2$	2.0
Norvaline	—$CH_2CH_2CH_3$	3.6×10^2
Norleucine	—$CH_2CH_2CH_2CH_3$	3.0×10^3
Phenylalanine	—CH_2—C$_6$H$_5$	1.0×10^5

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

Table 6-8
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-some enzymes have achieved an **astonishing state** of perfection!

-only way to **increase rate** is to (i) generate substrate at the active site or (ii) decrease the size of the E moiety

-first possibility arises when two or more E are combined in a multi-enzyme complex

-the product of one reaction can then be released close to the active site of the next E

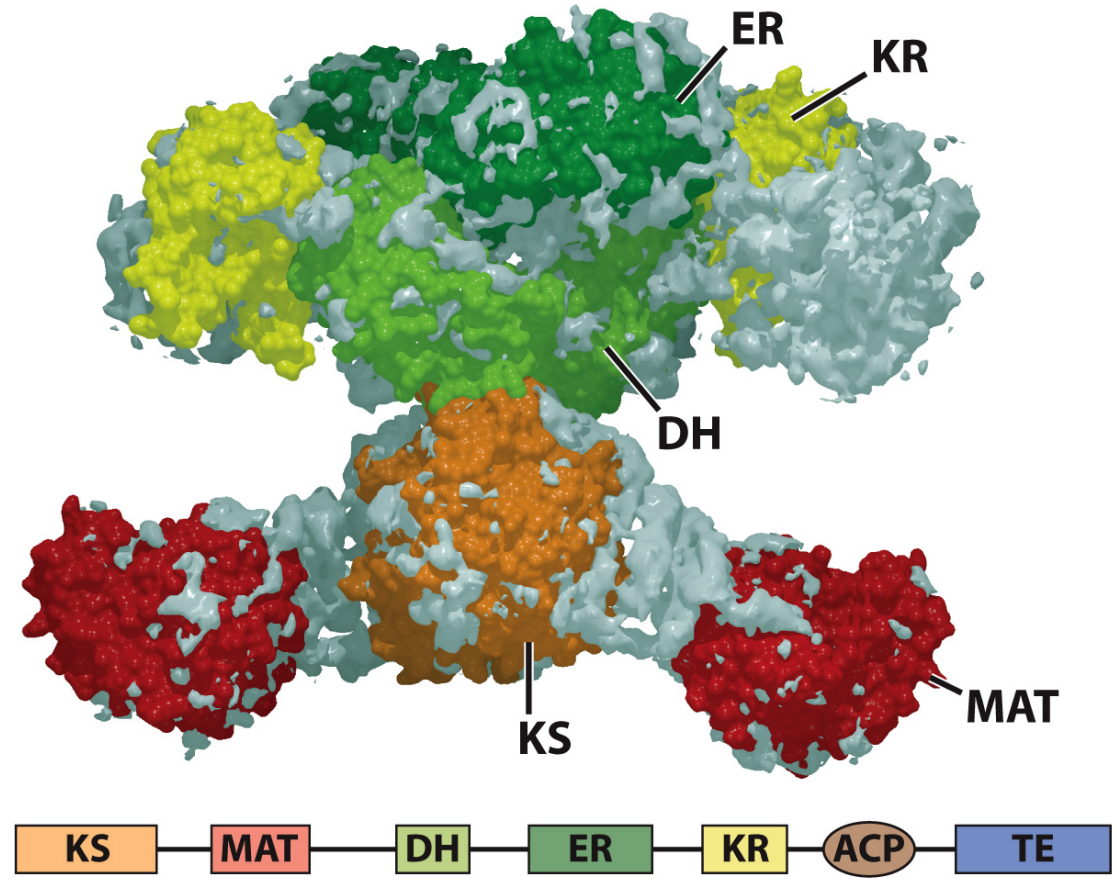

Figure 21-3a
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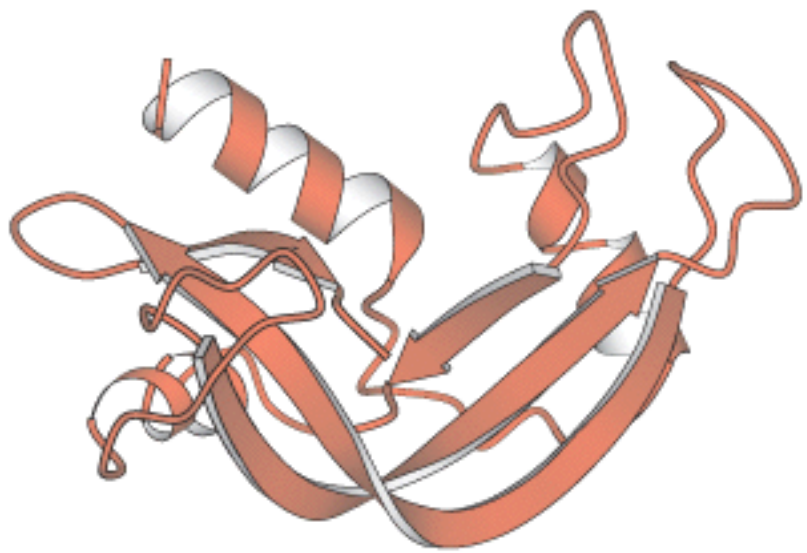
-second possibility is difficult since the sizes of E are limited by the need for tertiary structure to create the proper active site geometry

-large sizes of E are dictated by the need for secondary binding sites for effector molecules that regulate enzymatic activity



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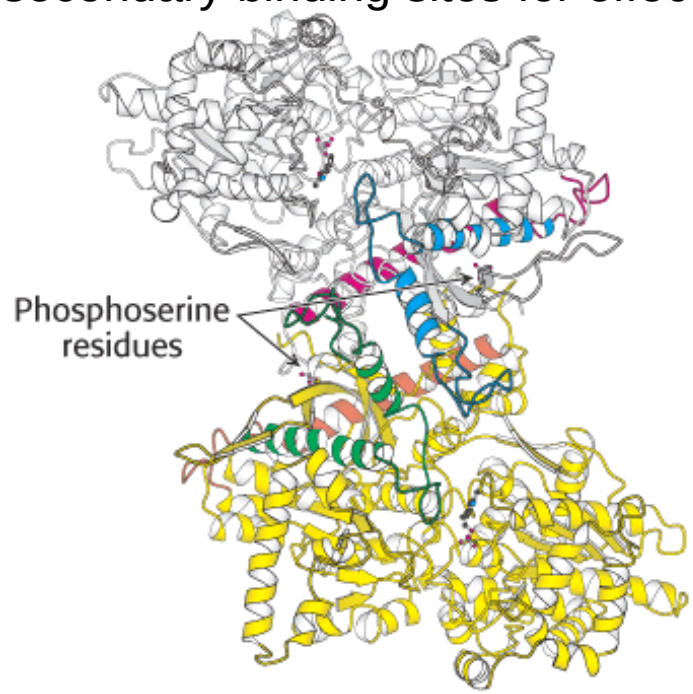
Human ribonuclease



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Phosphorylase *a* (in R state)

Appendix

Michaelis-Menten Equation (Integrated Form)

Comment: You may wish to use the integrated Michaelis-Menten equation to determine the precise changes in [S] during an enzyme-catalyzed reaction or to determine how long it will take to form a certain amount of product, etc. This derivation is for your reading pleasure only and is meant to be background information to support some questions found in Problem Set#2 (e.g., Question #8).

(1) Derivation

-for calculations involving changes as a function of reaction time, the integrated form of the Michaelis-Menten equation must be used

-the Michaelis-Menten equation is a differential equation since velocity is the rate of change of either substrate consumption or product formation

thus, $v = -d[S]/dt = d[P]/dt$

the M-M equation can be written as: $-d[S]/dt = V_{max}[S]/(K_M + [S])$ **[M-1]**

separating variables: $-V_{max}dt = (K_M/[S] + 1)d[S]$ **[M-2]**

equation can be integrated between an initial time (t_0) and a final time (t) for which the corresponding substrate concns are $[S]_0$ and $[S]_t$, respectively.

$$-1\int_{t0}^{t} V_{max}\,dt = \int_{t0}^{t}\left(\frac{K_M}{[S]}+1\right)d[S] \quad \textbf{[M-3]}$$

$V_{max}(t-t_0) = K_M\ln([S]_0/[S]_t) + ([S]_0 - [S]_t)$ **[M-4]**

If we let $t_0 = 0$ and $y = ([S]_0 - [S]_t)$ and substituting for $[S]_t$

where $[S]_t = [S]_0 - y$

then, $V_{max}t = K_M \ln([S]_0/[S]_0 - y) + y$ **[M-5]**

converting to ordinary logs: $V_{max}(t) = 2.303\ K_M \log([S]_0/([S]_0-y)) + y$ **[M-6]**

rearranging yields: $V_{max} = 2.303\ K_M\ (1/t) \log([S]_0/([S]_0-y)) + (y/t)$ **[M-7]**

$2.303\ K_M\ (1/t) \log([S]_0/([S]_0-y)) = V_{max} - (y/t)$ **[M-8]**

$2.303/t \log([S]_0/([S]_0-y)) = -1/K_M\ (y/t) + V_{max}/K_M$ **[M-9]**

(2) Simplified forms

-Equation M-9 (above) reduces to simpler forms when the substrate concn is either very large or small compared to K_M and y

-if $[S]_0 >> K_M$ and much larger than the change in substrate concn (y) then equation M-9 reduces to:

$$2.303/t \log([S]_0/[S]_0) = -1/K_M (y/t) + V_{max}/K_M \quad \textbf{[M-10]}$$

that is, $y/t = V_{max}$ **[M-11]**

but, y/t is the change in [S] per unit time, i.e., the velocity of the reaction

$$v = y/t = V_{max} \quad \textbf{[M-12]}$$

likewise, if $[S]_0$ (and hence y) is $<< K_M$ then equation M-9 reduces to:

$$2.303/t \log([S]_0/([S]_0-y)) = V_{max}/K_M \quad \textbf{[M-13]}$$

which describes a first order reaction (initial part of v versus [S] curve)

V_{max}/K_M is the first order rate constant (k) and thus equation M-13 can be rewritten:

$$2.303 \log([S]_0/[S]_t) = kt \quad \textbf{[M-14]}$$

$$\log([S]_0/[S]_t) = kt/2.303 \quad \textbf{[M-15]}$$

$$\log[S]_0 - \log[S]_t = kt/2.303 \quad \textbf{[M-16]}$$

$$-\log[S]_t = kt/2.303 - \log[S]_0 \quad \textbf{[M-17]}$$

$$\log[S]_t = -kt/2.303 + \log[S]_0 \quad \textbf{[M-18]}$$

$$[S]_t = [S]_0 e^{-kt} \quad \textbf{[M-19]}$$

the half-life for this first order reaction: at $t_{1/2}$ then $[S]_t/[S]_0 = 0.5$
so: $[S]_t/[S]_0 = e^{-kt}$ **[M-20]**

$$0.5 = e^{-kt} \quad \textbf{[M-21]}$$

$$\ln(0.5) = -kt_{1/2} \quad \textbf{[M-22]}$$

$$t_{1/2} = 0.693/k = 0.693K_M/V_{max} \quad \textbf{[M-23]}$$