Enzyme Kinetics and Inhibition

Stryer Short Course
Chapter 7

Enzyme Kinetics

- How fast an enzyme catalyzed reaction goes
- Why study enzyme kinetics?
  - Helps us understand mechanism of enzyme (how it works)
  - Investigation of mutations in metabolic pathways
  - Understanding of regulation of biochemical reactions (up or down regulation of catalyst)

Simple Mechanisms

- Chemical mechanism

$$\text{Substrate} \longrightarrow \text{Product}$$

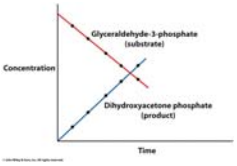
- Enzyme Catalyzed

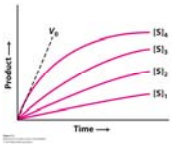
$$\text{Substrate} + \text{Enzyme} \rightleftharpoons \text{Reactive Complex} \longrightarrow \text{Product} + \text{Enzyme}$$

- How do we measure kinetics experimentally?

Chemical Kinetics

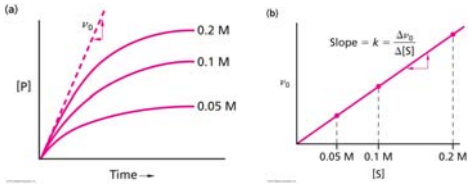
- Rate: measure product formed per second
- Rate slows as reactant disappears
- Measure initial rate
- Do a second experiment with more starting material, and the initial rate is faster





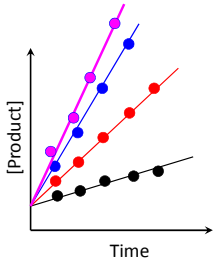
Chemical Kinetics

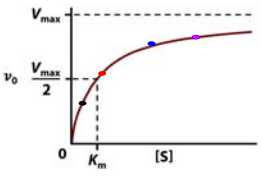
- Secondary plot: change in rate as a function of how much substrate you started with
- Linear plot—does that make sense?

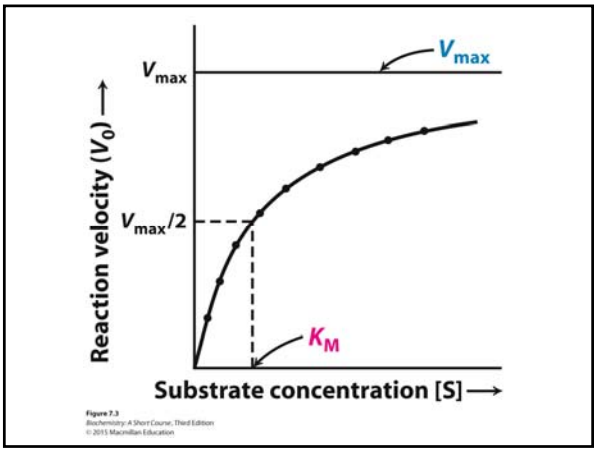


Enzyme Kinetics

- Keep the [E] constant and low, and test how changing the [S] affects initial rates (physiologically relevant)
- Called Michaelis-Menton Treatment

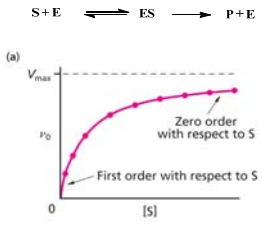






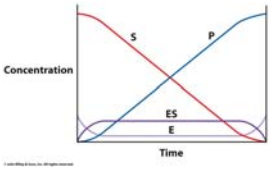
Interpretation of Shape

- Low [S]
  - Rate very dependent on [S]
  - Binding is rate limiting
- High [S]
  - Rate independent
  - Saturation of E
  - Chemistry is rate limiting



Mechanism and Assumptions

- E + S ←→ ES →E + P
  - Low [E] relative to [S]
    - Steady state
  - Initial rates
    - No back rxn
    - No pdt inhibition
  - **Can derive a rate equation**

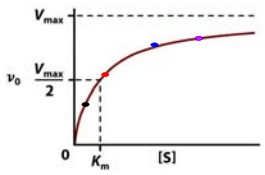


Michaelis-Menton Kinetics

- Rectangular hyperbola
- Parameters

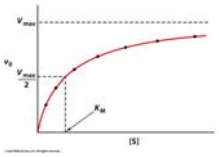
$$y = \frac{ax}{b + x}$$

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$



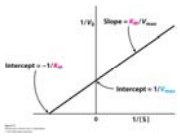
Graphical Determination of Kinetic Parameters

- Analyze hyperbola
- Construct linear plot
- Double reciprocal



Lineweaver–Burk equation:

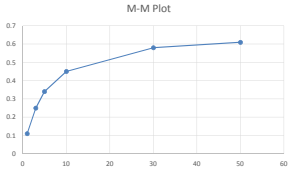
$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

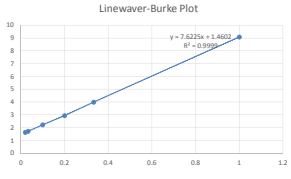


Problem 7

- Determine kinetic parameters Vmax and Km:

[S] mM	[P] at 1 min (nM)
1	0.11
3	0.25
5	0.34
10	0.45
30	0.58
50	0.61





Maximum Velocity and the Catalytic Constant

- What two things contribute to the maximum velocity limit?
  - Amount of enzmye
  - Chemical ability of enzyme (catalytic constant)
- $V_{max} = [E]\ k_{cat}$
- Only k_{cat} tells us about the enzyme
  - Maximum # of substrate molecules per active site per second
  - Turnover number

Table 7.2 Turnover numbers of some enzymes

Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Michaelis Constant

- K_m is the [S] at which the reaction reaches half its maximum velocity
- Physical meaning (assuming equilibrium binding): K_m is the dissociation constant for ES
- K_m is [S] at which enzyme is half-bound
- K_m is measure of affinity of enzyme for S
- Low K_m is tight binding

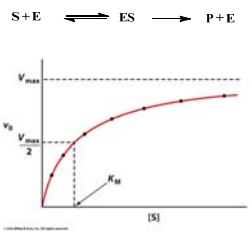


Table 7.1 K_M values of some enzymes

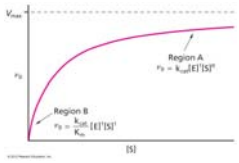
Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50

Enzyme Efficiency

- At low [S], the second order rate constant is k_{cat}/K_m
- Efficient enzymes have large k_{cat}/K_m
  - Large k_{cat} and/or
  - Small K_m
- Catalytic perfection at 10^8 or 10^9 M^{-1} S^{-1}
- Diffusion control

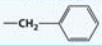
$$v = \frac{k_{cat}[E][S]}{K_m + [S]}$$

Assume large [S] and small [S]



Also Called Specificity Constant

Table 7.3 Substrate preferences of chymotrypsin

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

Source: Data from A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W.H. Freeman and Company, 1999), Table 6.3.

Table 7.3
Biochemistry: A Short Course, Third Edition

Case Study:

Table 7.4 Enzymes for which k_{cat}/K_M is close to the diffusion-controlled rate of encounter

Enzyme	k_{cat}/K_M (s^{-1} M^{-1})
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β-Lactamase	1×10^8
Superoxide dismutase	7×10^9

Source: Data from A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 4.5.

Table 7.4
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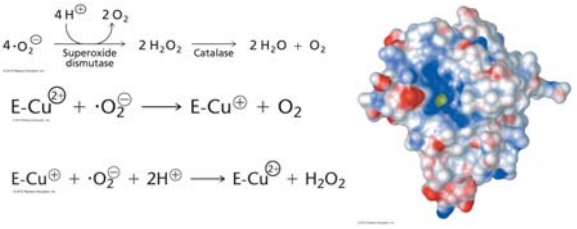
Superoxide Dismutase: Better than Diffusion!



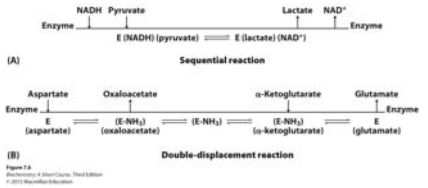
Catalytic Proficiency

Table 5.2 Catalytic proficiencies of some enzymes

	Nonenzymatic rate constant (k_n in s^{-1})	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)	Catalytic proficiency
Carbonic anhydrase	10^{-1}	7×10^{6}	7×10^{7}
Chymotrypsin	4×10^{-9}	9×10^{7}	2×10^{16}
Chorismate mutase	10^{-5}	2×10^{6}	2×10^{11}
Triose phosphate isomerase	4×10^{-6}	4×10^{8}	10^{14}
Cytidine deaminase	10^{-10}	3×10^{6}	3×10^{16}
Adenosine deaminase	2×10^{-10}	10^{7}	5×10^{16}
Mandelate racemase	3×10^{-13}	10^{6}	3×10^{18}
β-Amylase	7×10^{-14}	10^{7}	10^{20}
Fumarase	10^{-13}	10^{9}	10^{21}
Arginine decarboxylase	9×10^{-16}	10^{6}	10^{21}
Alkaline phosphatase	10^{-15}	3×10^{7}	3×10^{22}
Orotidine 5′-phosphate decarboxylase	3×10^{-16}	6×10^{7}	2×10^{23}
Uroporphyrinogen decarboxylase	10^{-17}	2×10^{7}	2×10^{24}

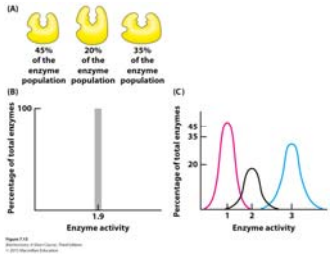
Non-MM Kinetics

- Multi-substrate
  - Each substrate has its own K_M
  - Random, ordered, ping-pong
  - k_{cat} not simplified to k_2



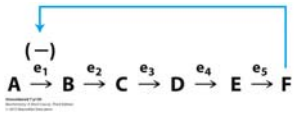
Other Kinetics Experiments

- Stopped-flow kinetics
- Single molecule studies



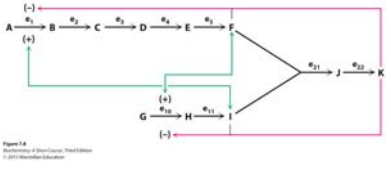
Allosteric Regulation

- Feedback Inhibition
  - Inhibitors don't resemble substrates or products
  - Bind to "other" site
  - "Allosteric enzymes always catalyze the committed step of a metabolic pathway"



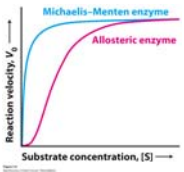
Positive Effector

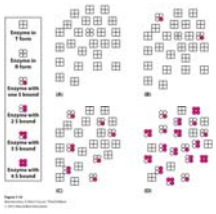
- Example: branched system
- In addition to inhibition, need stimulatory molecule



Mechanism of Allosteric Enzymes

- Concerted Model of Cooperativity

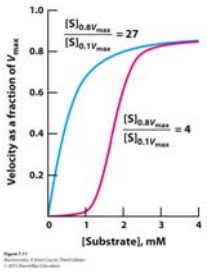




- Sequential Model

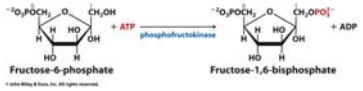
Physiological Impact

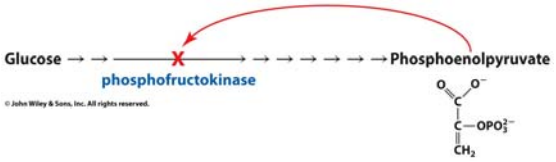
- Steeper slope over smaller range of substrate change
- Greater sensitivity to change in [S]
- Greater impact of regulator molecules



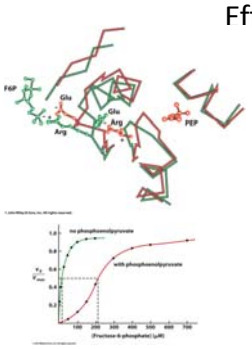
Example: Feedback Inhibition

- Glucose metabolism:





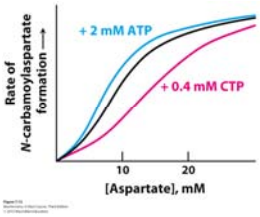
Molecular Mechanism of Allosteric Fffect



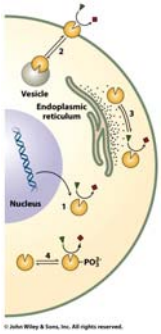
- PEP binding in allosteric site causes conformational shift in neighbor
- An Arg essential for F6P binding is replaced with Glu
- T vs. R state
- Cooperative, no effect on V_{max}, but only apparent K_M

Problem

- ATP and CTP are allosteric effectors of aspartate transcarbasmoylase. Refer to the graph to explain with is an inhibitor and which is a positive effector.



Other Modes of Regulation



- Transcriptional level
- Compartmentalization
- Intracellular signal
- Covalent modification