Computational Systems Biology

Lecture 3: Enzyme kinetics

Fri 19 Jan 2009

Images from:
D. L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.
A. Cornish-Bowden Fundamentals of Enzyme Kinetics, Portland Press, 2004
A. Cornish-Bowden Enzyme Kinetics, IRL Press, 1988

Summary:

- Simple enzyme kinetics
- Steady-state rate equations
- Reactions of two substrates
- Inhibition of enzyme activity
- pH dependence
- Biological regulation of enzymes

Simple Enzyme Kinetics

Basics

- Enzyme kinetics studies the reaction rates of ***enzyme-catalyzed*** reactions and how the rates are affected by changes in experimental conditions

- An essential feature of enzyme-catalyzed reactions is ***saturation***: at increasing concentrations of substrates the rate increases and approaches a limit where there is no dependence of rate on concentration (see slide with limiting rate V_{max})

- Leonor Michaelis and Maud Menten were among the first scientist to experiment with enzyme kinetics in a “modern” way, controlling the pH of the solution etc.

- The convention used for this slides is to use UPPERCASE for the molecular entity: e.g. E is an enzyme molecule and *italics lowercase* for the concentration: e.g. e_0 is the enzyme concentration at time zero (initial concentration). Also square brackets can be used for concentration, e.g. [E] = enzyme concentration.

For additional material: Fundamentals of Enzyme Kinetics, Athel Cornish-Bowden, 2004 or Enzyme Kinetics, Athel Cornish-Bowden and C. W. Wharton, IRL Press, 1988

A simple view: E+A = EA as an equilibrium

- The mechanism: the first step of the reaction is the binding of the substrate (A) to the enzyme (E) to form and enzyme-substrate complex (EA) which then reacts to give the product P and free enzyme E
- The concentrations: the total initial enzyme concentration is e_0 , and the complex concentration is x. The substrate (A) concentration should too be $(a_0 - x)$, but since the substrate concentration is usually very high:
- The conversion is considered an equilibrium with equilibrium constant K_s
- The slowest step of the reaction is the EA to E+P and hence the dominant rate for all the reaction is the rate $v = k_0 x$ or, considering the form 3.

$$\begin{array}{ccccccccc} 1. & E & + & A & \underset{K_s}{=} & EA & \xrightarrow{k_0} & E & + & P \\ & (e_0 - x) & & a & & x & & & & p \end{array}$$

$$a \cong a_0 \cong (a_0 - x)$$

$$2.\ K_s = \frac{[E][A]}{[EA]} = \frac{(e_0 - x)a}{x} \quad \text{or} \quad 3.\ x = \frac{e_0 a}{(K_s + a)}$$

$$4.\ v = \frac{k_0 e_0 a}{(K_s + a)}$$

Steady-state rate equations

The steady-state assumption

- When the enzyme is first mixed with a large excess of substrate there is an initial period, lasting just a few microseconds, the *pre-steady state*, during which the EA complex concentration builds up

- The reaction quickly achieves a steady state in which [ES] remains approximately constant over time (Briggs and Haldane, 1925)

For additional material: Fundamentals of Enzyme Kinetics, Athel Cornish-Bowden, 2004 or Enzyme Kinetics, Athel Cornish-Bowden and C. W. Wharton, IRL Press, 1988

A more general view: E+A ←→ EA as reversible

- The mechanism: the first step is now reversible with a forward (k_1) and a backward (k_{-1}) constant
- The rate of change of the concentration of intermediate (dx/dt) is the difference between the rate at which it is produced from E + A and the sum of the rates at which it is converted back into free E and A and forward into free E and P
- Briggs and Haldane postulated that dx/dt should be positive at the instant of mixing of E with A (because at the beginning there is no EA and then it builds up) but then the removal rate of EA would rapidly increase, until it balances the rate of production. This is the *steady-state*

5. $$\underset{(e_0 - x)}{E} + \underset{a}{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{x}{EA} \xrightarrow{k_0} E + \underset{p}{P}$$

6. $$\frac{dx}{dt} = k_1(e_0 - x)a - k_{-1}x - k_2x$$

7. $$\frac{dx}{dt} = k_1(e_0 - x)a - k_{-1}x - k_2x = 0$$

The Michaelis-Menten equation:

effects of substrate concentration on a reaction initial velocity

- Rearranging equation 7 we obtain:

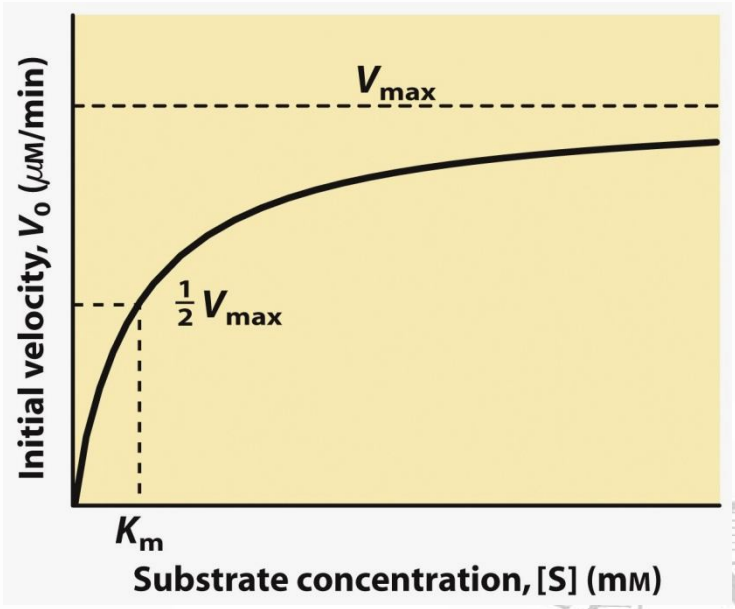
$$8.\quad v = \frac{k_0 e_0 a}{K_m + a}$$

- the Michaelis-Menten equation, where

$$K_m = \frac{k_{-1} + k_0}{k_1}$$

- K_m (the Michaelis constant) is visible in the diagram as the concentration of substrate at which the initial velocity is half of the maximum velocity

- *You can experiment with this equation on: http://bcs.whfreeman.com/lehninger/ clicking on → Chapter 6: Enzymes → "Living graphs" menu*



Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

The Michaelis-Menten equation:

effects of substrate concentration on a reaction maximum velocity

- When *a* is much smaller than K_m it can be ignored giving:

$$9.\quad v = \left(\frac{k_0}{K_m}\right) e_0 a$$

with first-order dependences on both enzyme and substrate, or second-order kinetics overall.

- $\mathbf{k_0/K_m}$ is thus called the *second order rate constant* or, more importantly, the *specificity constant*, as it is specific for each enzyme type.

- As *a* increases and surpasses K_m making it insignificant, 8. becomes:

$$10.\quad v \cong k_0 e_0 = V$$

- Where V is the *limiting rate* (better definition than "V_{max}", since the value is approached rather slowly, and never really reached)

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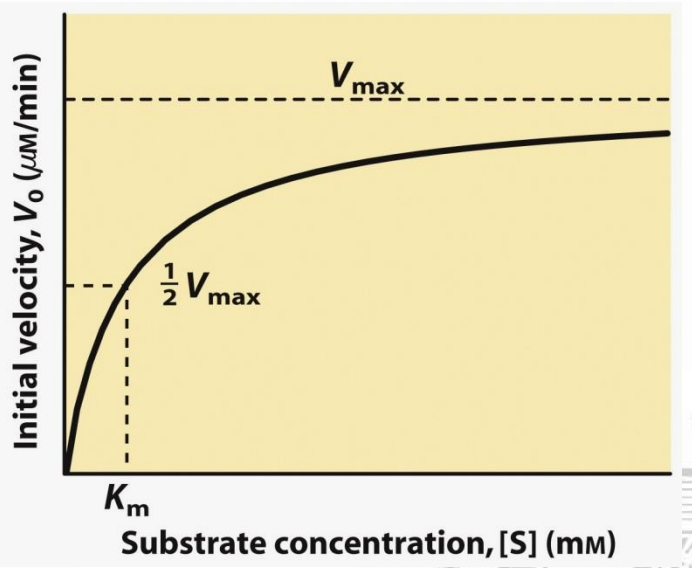

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Lineweaver-Burk equation

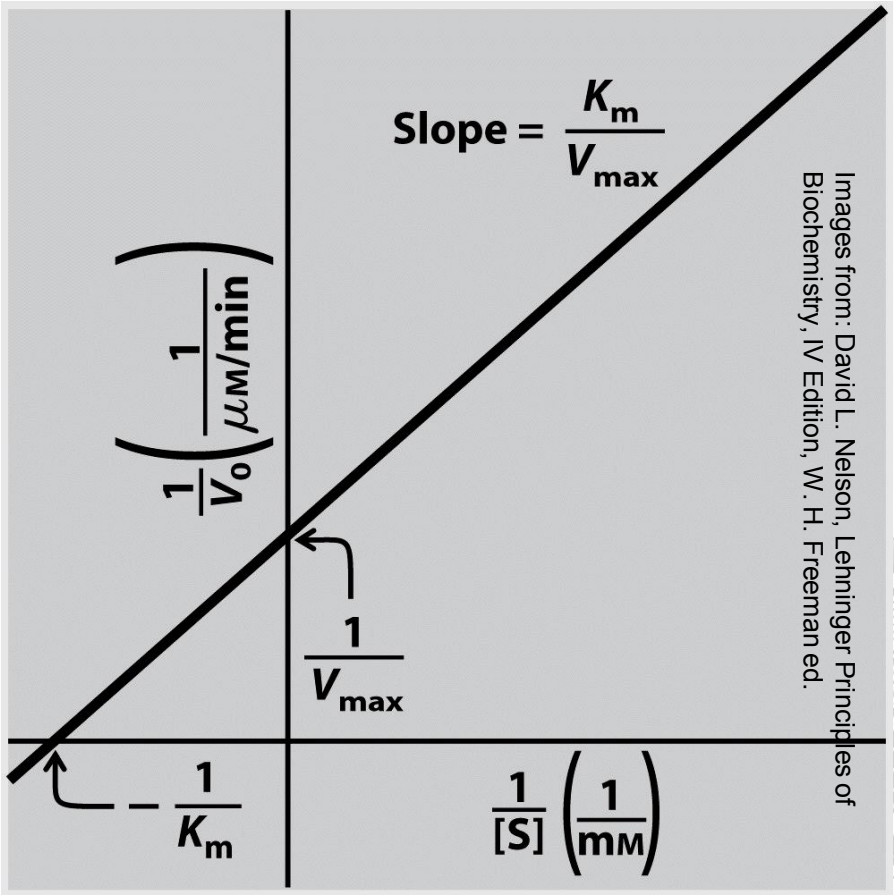
- The Lineweaver-Burk equation

$$11.\quad \frac{1}{v} = \frac{1}{V} + \frac{K_m}{Va}$$

is a rewriting of the Michaelis-Menten equation that is often used for the *(not very accurate)* determination of kinetic parameters from the plot.

- It is useful for analysis of multi-substrate and inhibited enzymatic reactions (see next slides)
- *You can experiment with this equation on: http://bcs.whfreeman.com/lehninger/ clicking on → Chapter 6: Enzymes → "Living graphs" menu*

Double reciprocal plot:



Experimental investigation of fast reactions

- *Thought natural chemical reactions were fast?* (e.g. interconversion of bicarbonate and dissolved carbon dioxide) Think again…Enzymes like carbonic anhydrase in your lungs can accelerate the reaction of a 10^7 factor, processing a staggering 400000 molecules of HCO_3^- per second. The enzyme converts the molecules almost as fast as they can diffuse in acqueous solution. Biochemists call it reaching "catalytic perfection".

How can we study such a fast process?

- Flow methods: involves a continuous flow of enzyme and substrate through a mixing chamber in a long tube. Observation at a certain distance gives a population of molecule of a fixed "age" from the instant of mixing. This allows reactions with a half-time of milliseconds to be studied with equipments requiring several seconds for each measurement.
- Stopped flow methods: similar, but with a mechanical syringe stopping the flow almost instantaneously. Or involving the addition of a quenching (stopping) agent (e.g. trichloroacetic acid that destroys the enzyme) or bringing the mixture to a very low temperature with liquid nitrogen, so as to "freeze" the situation at a given instant.
- Perturbation methods: for extremely quick reactions the mixture already at steady state is given a temperature-jump of +10 °C with an electric current. The steady-state rate will not be same at the higher temperature and the "relaxation" to the new equilibrium is observed.

From: Cornish-Bowden, Enzyme Kinetics, 1988 - IRL press and Nelson, Lehninger Principles of Biochemistry, IV Ed.,chapter 6.

Reactions of two substrates

Types of enzyme mechanism for reactions of two substrates

- The most common enzyme mechanism involves a chemical group transfer from one substrate to another.

There are multiple possibilities to be considered:

(A)

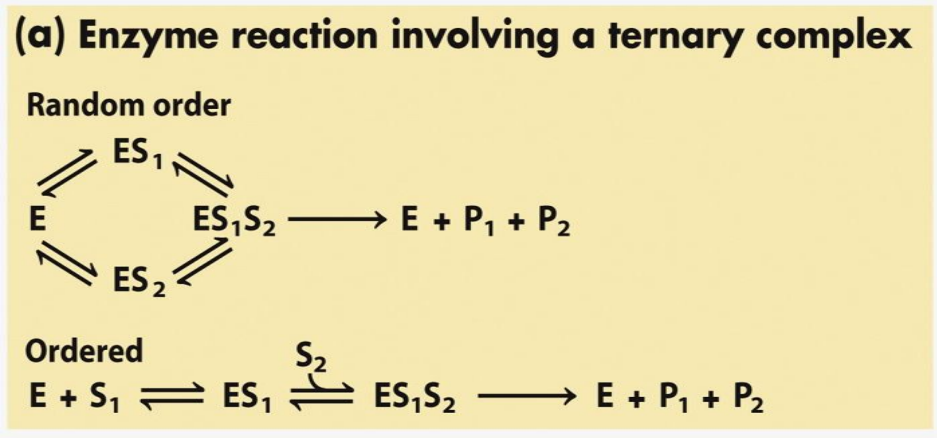
1. Does the reaction involve transfer of the group from the donor (first substrate) to the enzyme, followed by a second transfer from the enzyme to the acceptor (second substrate)? [*Ping-Pong mechanism]*
2. Or does the transfer occur in a single step while both donor and acceptor are still in the active site of the enzyme? [*Ternary complex]*

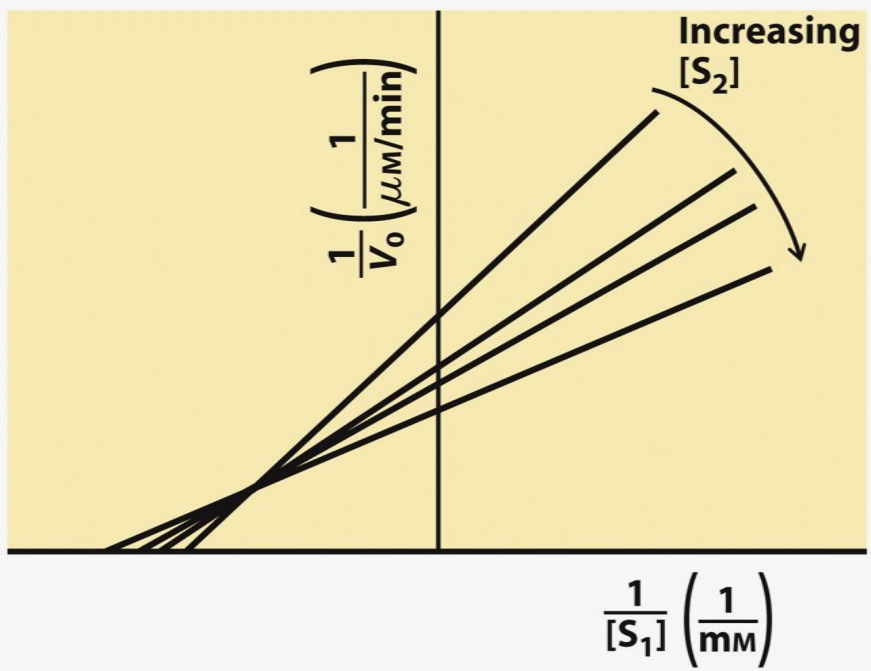
(B)

1. In this second case, is the order of binding of donor and acceptor random? [*Random-order*]
2. Or is it compulsory that one of the substrates enters the active site first? [*Compulsory-order*]

- And, can we distinguish the mechanism from the kinetics properties of the enzyme? Yes.

Two substrates mechanism: Ternary complex





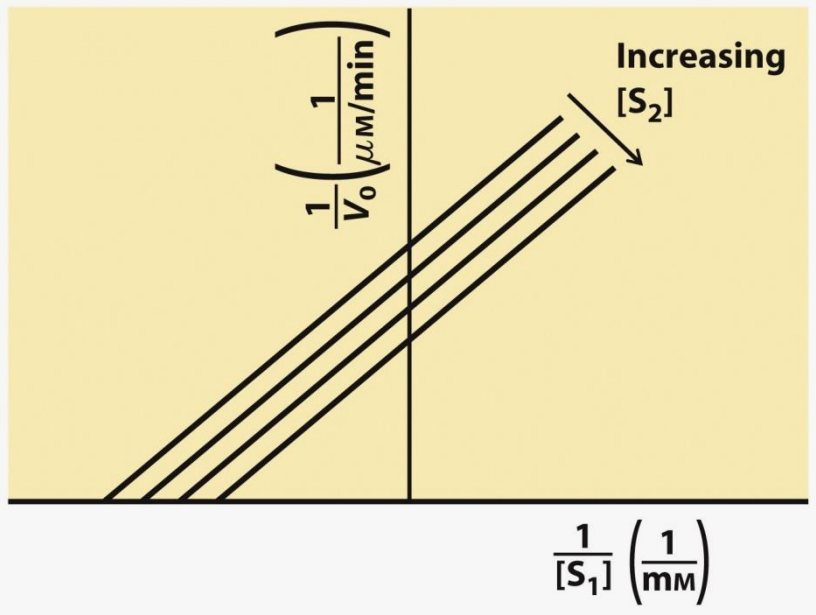
- Intersecting of the lines at increasing concentrations of the second substrate ([S_2])indicates a ternary complex

Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

Two substrates mechanism: Ping-Pong

(b) Enzyme reaction in which no ternary complex is formed

$$E + S_1 \rightleftharpoons ES_1 \rightleftharpoons E'P_1 \overset{P_1}{\rightleftharpoons} E' \overset{S_2}{\rightleftharpoons} E'S_2 \longrightarrow E + P_2$$



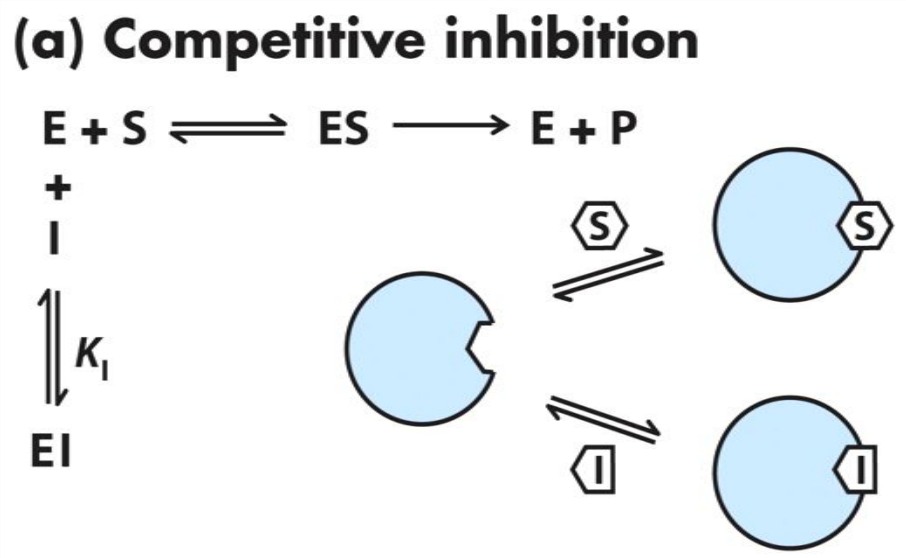
- Parallel lines at increasing concentrations of the second substrate ([S_2])indicates a Ping Pong mechanism.

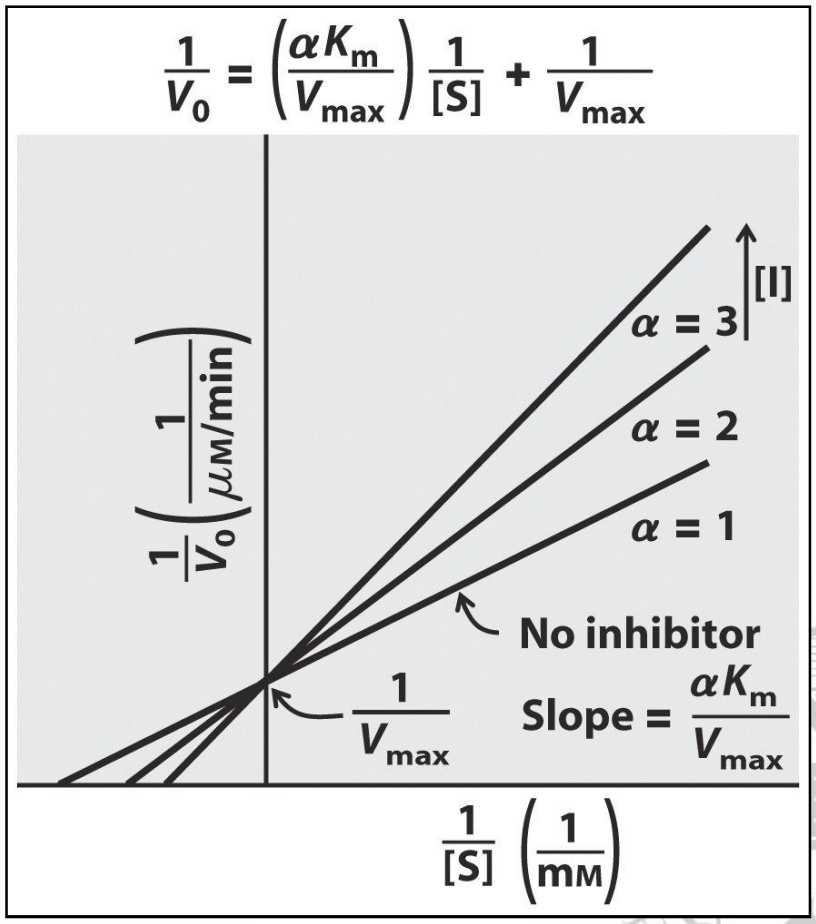
Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

Reversible inhibition of enzyme activity

Competitive Inhibition

- A competitive inhibitor (I) is a substance that competes with the substrate for the same active site
- αK_m = "apparent" constant when the inhibitor is present





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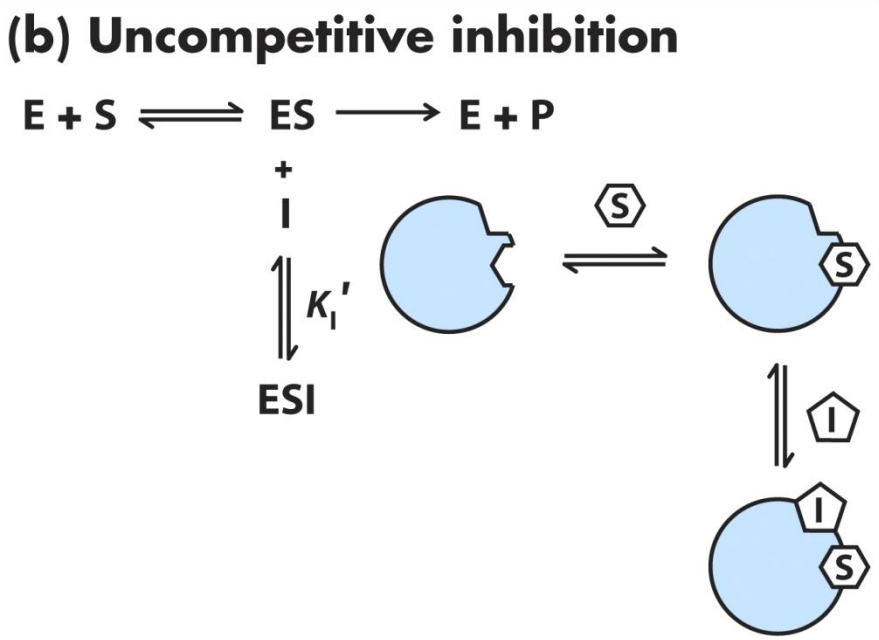
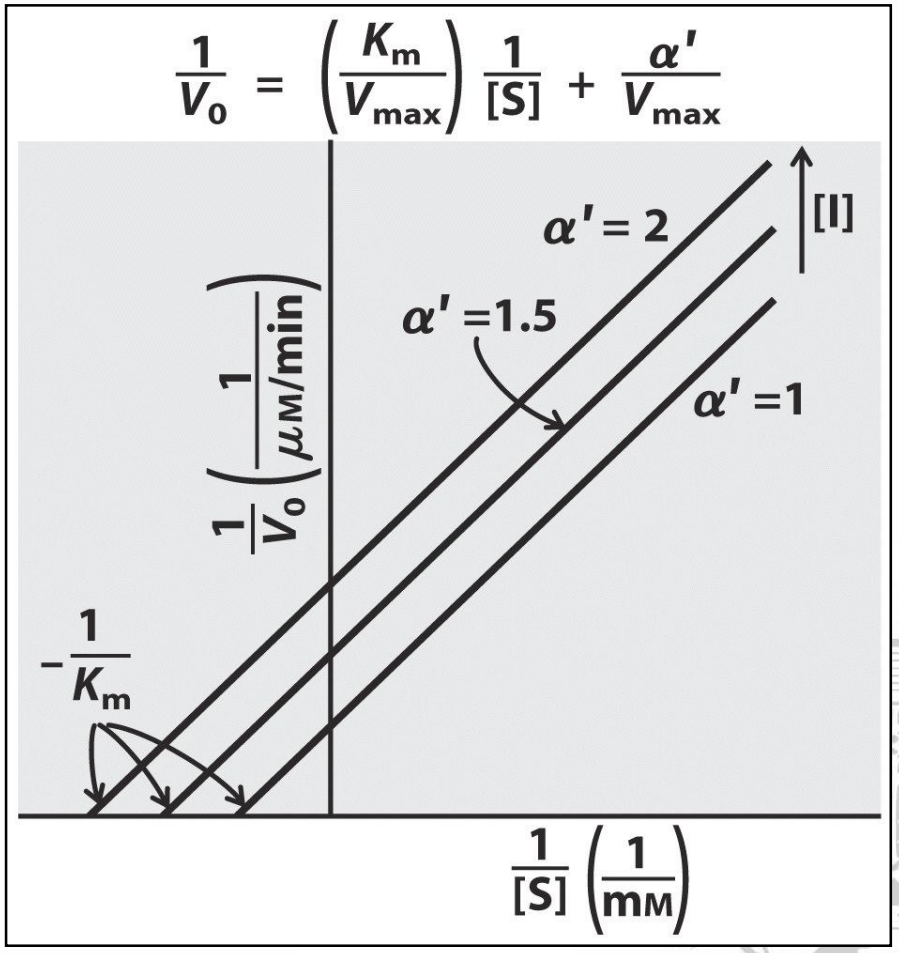
Uncompetitive Inhibition (not very common)

- An uncompetitive inhibitor (I) binds at a site distinct from the substrate active site





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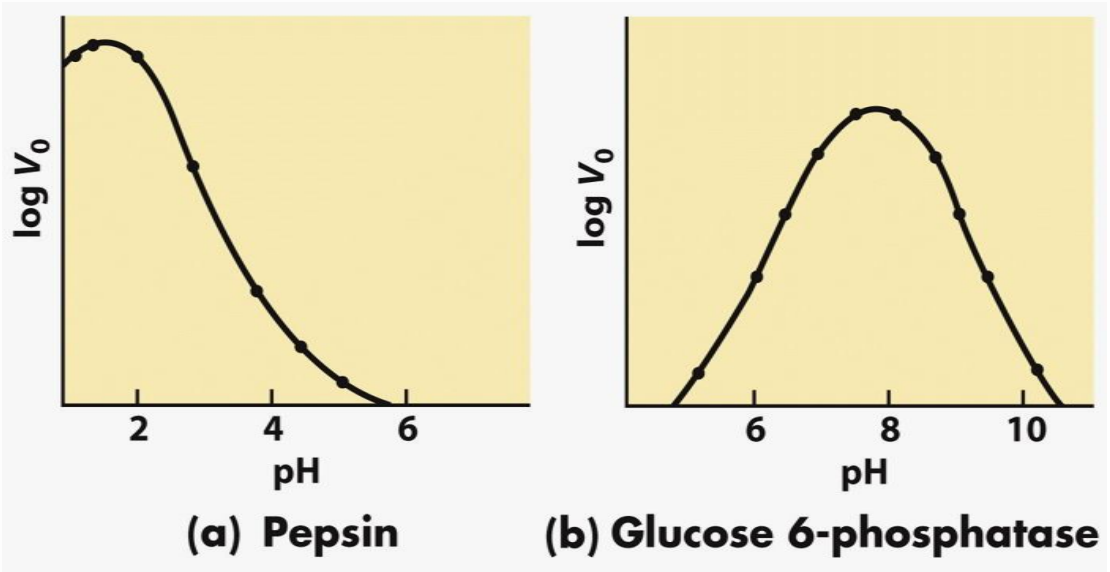
pH dependence of enzyme activity

pH activity profiles

- The plots are in a logarithmic scale (pH is a logarithmic scale of proton concentration [H^+]) and have been generated monitoring the initial reaction velocity in buffers (acqueous solutions) of different acidity.
- The pH optimum for enzyme activity is generally close to the pH of the environment/cell location natural for the enzyme
- Pepsin (a) is a stomach enzyme that hydrolyzes peptide bonds allowing the digestion of proteins (e.g. in meat and fish). It has a very acidic optimum of pH 1.6, similar to the stomach environment.
- Glucose 6-phosphatase (b) in hepatocytes (liver cells) helps in regulating glucose release and has a more neutral optimum of pH (around 7).



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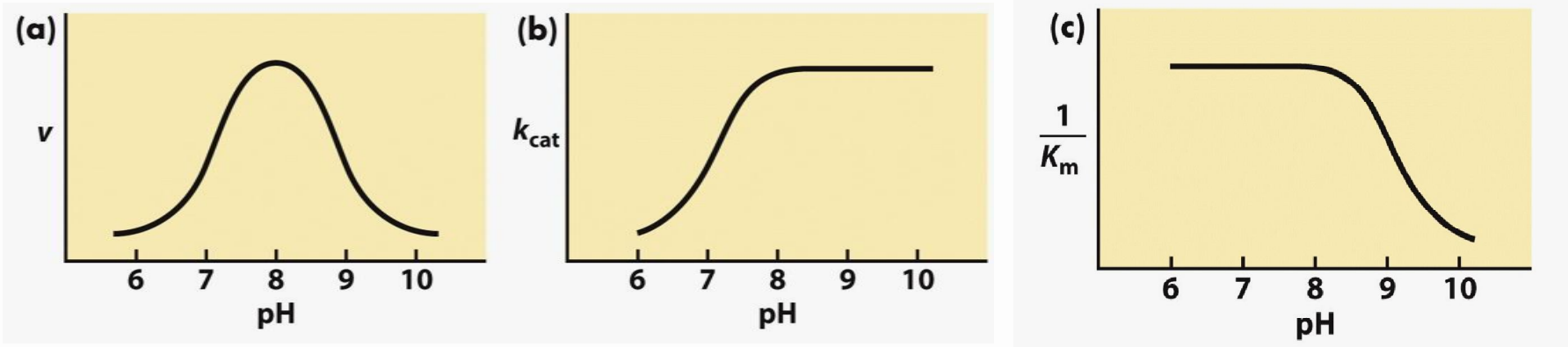
pH dependence for chymotrypsin

- Chymotrypsin (another enzyme that hydrolyzes peptide bonds) has an optimum of activity at pH 8
- The activity alteration is linked to the ionization state of the aminoacid side chains in the active site.
- The transitions in (b) at pH 7.5 and (c) at pH 8.5 are due to protonation of His^{57} (that should be unprotonated for best activity – 57 is the position along the chain of the enzyme protein) and unprotonation of Ile^{16} (that has to be protonated for best activity).



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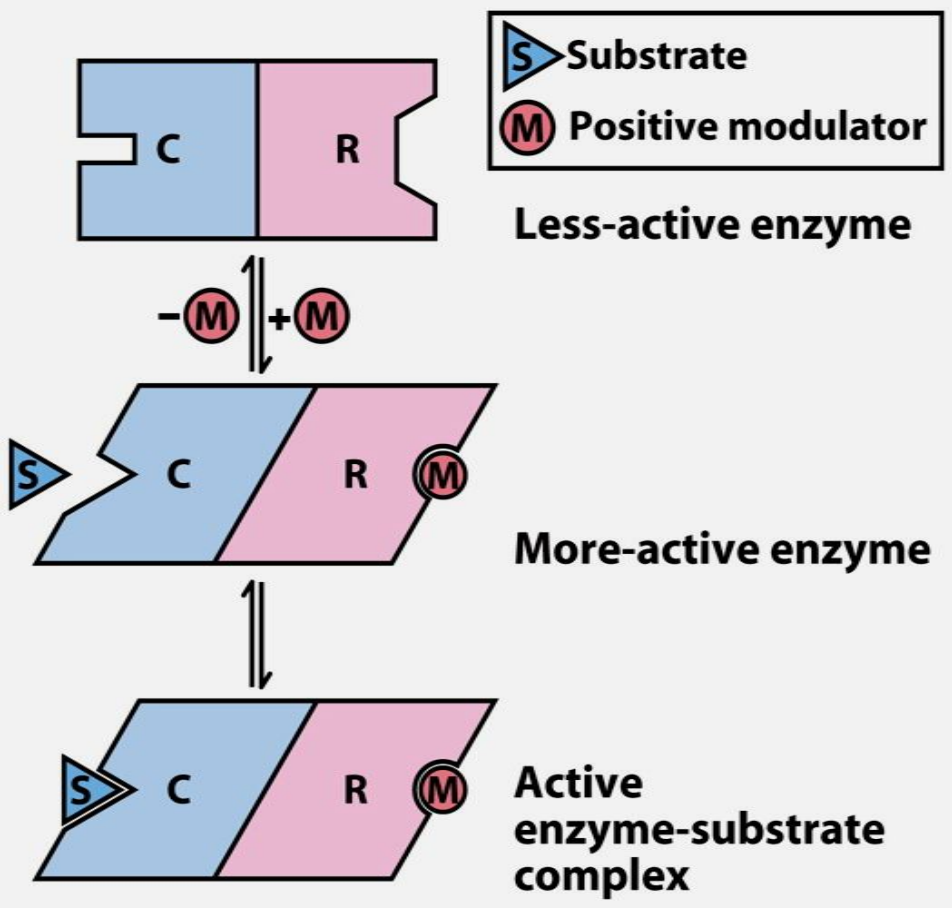
Biological Regulation

- Allosteric enzymes
- Feedback inhibition

Allosteric enzymes

- Allosteric enzymes tend to be multi-sub unit proteins
- The reversible binding of an allosteric modulator (here a positive modulator M) affects the substrate binding site



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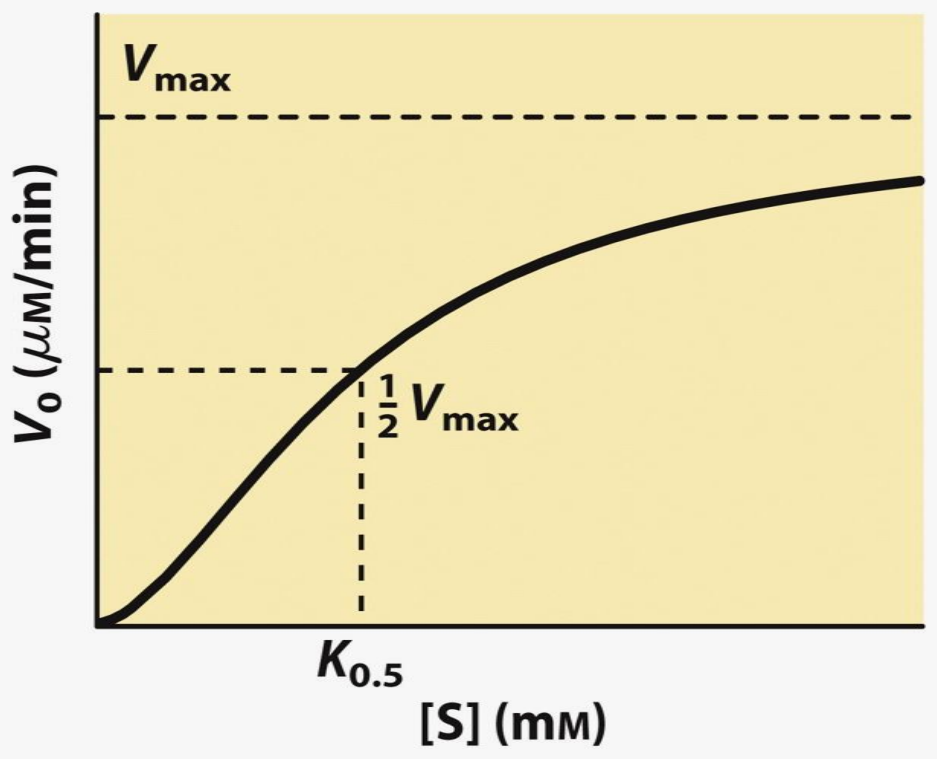
Sigmoid kinetic

- Allosteric enzymes do not show a Michaelis-Menten relationships between [S] and V_0
- The sigmoid kinetic behaviour reflects the cooperative interactions between multiple protein subunits
- The plot presented shows an enzyme in which the substrate S is also a positive regulator (homotropic enzyme)



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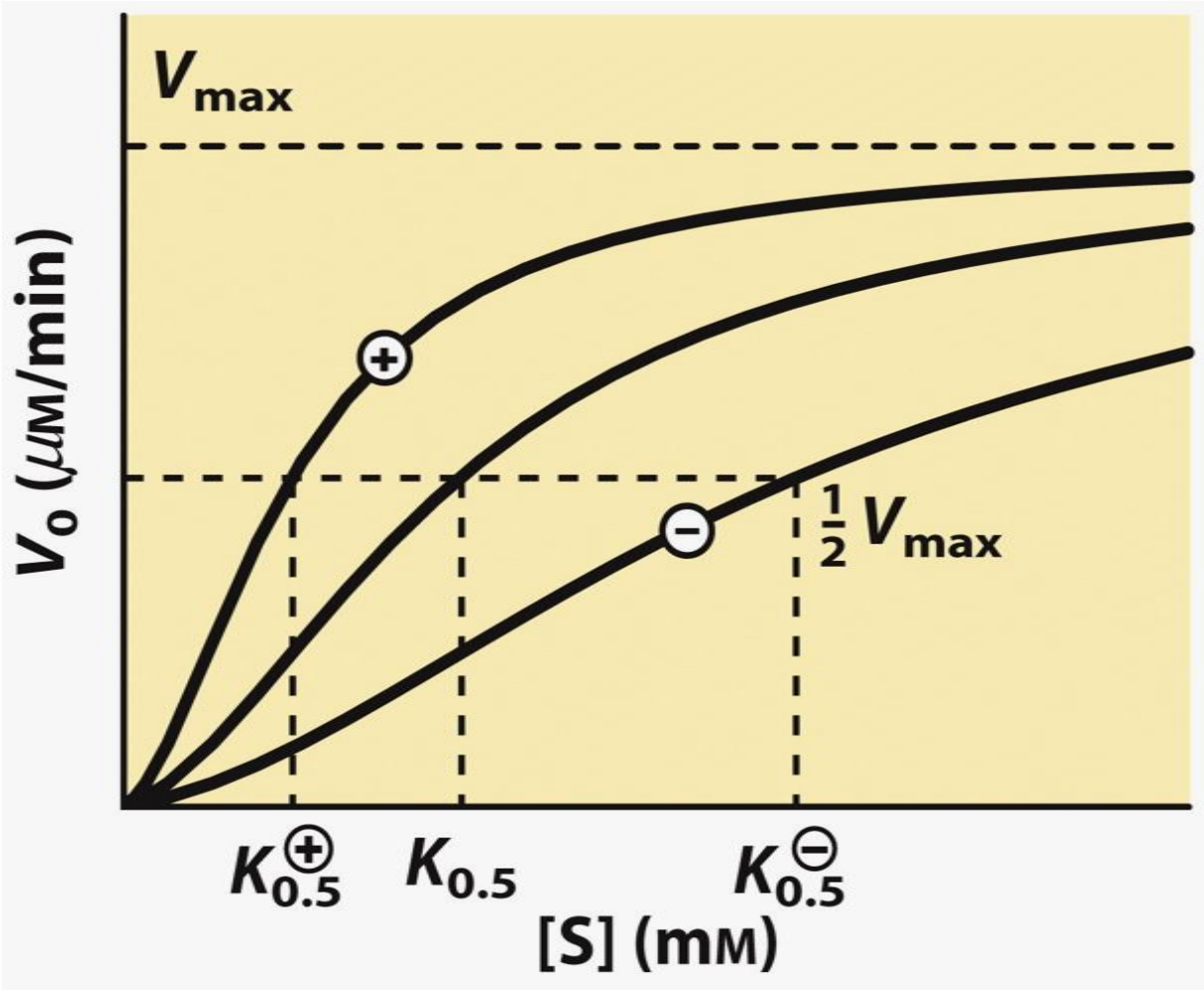
- Effects of a positive (+) and a negative (-) modulator that alter the $K_{0.5}$ without altering the maximum velocity V_{max}



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Feedback inhibition

Feedback in a metabolic pathway:

- L-Isoleucine, the product of the last enzyme of the pathway (E_5) inhibits the allosteric enzyme E_1 (threonine dehydratase) at the beginning of the pathway
- While the L-Isoleucine concentration decreases, the rate of the threonine dehydratase E_1 activity starts increasing again, producing new L-Isoleucine