Michaelis-Menten kinetic theory of enzyme action

1. **Effect of enzyme concentration on reaction velocity.**

If the substrate concentration is held constant, the velocity of the reaction is proportional to the enzyme concentration.

2. **Effect of substrate concentration on reaction velocity**

   a. When the substrate concentration ([S]) is low, the reaction velocity (v) is **first-order** with respect to substrate (i.e., v is directly proportional to [S]).

   b. At high substrate concentration, the reaction is **zero-order** (i.e., v is independent of [S]).

   c. At mid-[S], the reaction is mixed-order (i.e., the proportionality is changing).

Michaelis-Menten kinetic model.

An enzyme-catalyzed reaction involves the reversible formation of an enzyme-substrate complex [ES], which breaks down to form free enzyme [E] and product [P].

$$E + S \underset{k_1}{\overset{k_2}{\leftrightarrow}} ES \xrightarrow{k_3} E + P$$

k_1 is the rate constant for ES formation,

k_2 is the rate constant for the dissociation of ES back to E + S,

k_3 is the rate constant for the dissociation of ES to E + P.

The Michaelis-Menten equation

a. **The relationship of substrate concentration to velocity** for many enzymes may be described by equation (2), where v is the initial velocity of the reaction, $V_{max} = k_3[E]_T$, and $K_m = (k_2 + k_3)/k_1$. E_T is the total [E] present. [It should be noted that capital V is used only with the abbreviation for the maximum reaction velocity (V_{max}).]

$$v = \frac{V_{max}[S]}{([S] + K_m)}$$

b. **The Michaelis-Menten equation is based on three key assumptions.**

(1) [S] is very large compared with [E], so that when all E is bound in the form ES, there is still an excess of S.

(2) Only initial velocity conditions are considered. Thus, there is very little accumulation of P, and the formation of ES from E + P is negligible.

(3) Steady-state assumption. The rate of breakdown of ES equals the rate of formation of ES.

USING THE MICHAELIS-MENTEN EQUATION

1. Significance of the Michaelis constant (K_m)

$$v = \frac{V_{max}[S]}{([S] + K_m)}$$

a. If K_m is set equal to [S] and substituted into equation, then $v = {}^1/_2 V_{max}$. Therefore, K_m is equal to the substrate concentration at which the velocity is half-maximal.

b. K_m is not a true dissociation constant, but it does provide a measure of the affinity of an enzyme for its substrate. The lower the value of K_m, the greater the affinity of the enzyme for enzyme-substrate complex formation.

2. Lineweaver-Burk linear transform. Because it is difficult to estimate V_{max} from the position of an asymptote, as in the plot of a rectangular hyperbola, this linear transform of the Michaelis-Menten equation is often used.

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} * \frac{1}{[S]}$$

3. Graphical analysis.

Figure shows the straight-line graph obtained by plotting 1/v against 1/[S],

The y-intercept = $1/V_{max}$, the x-intercept = $-1/K_m$, the slope = K_m/V_{max}

ENZYME INHIBITION

Reversible inhibition. Different types of reversible enzyme inhibition are easily distinguished by analysis of Lineweaver-Burk plots.

1. Competitive inhibition

- **a.** Inhibitors compete directly with substrate for binding to the active site (i.e., the catalytic site).
- **b.** For a competitive inhibitor, the inhibition constant [K_i] is defined as the dissociation constant for the enzyme-inhibitor complex.

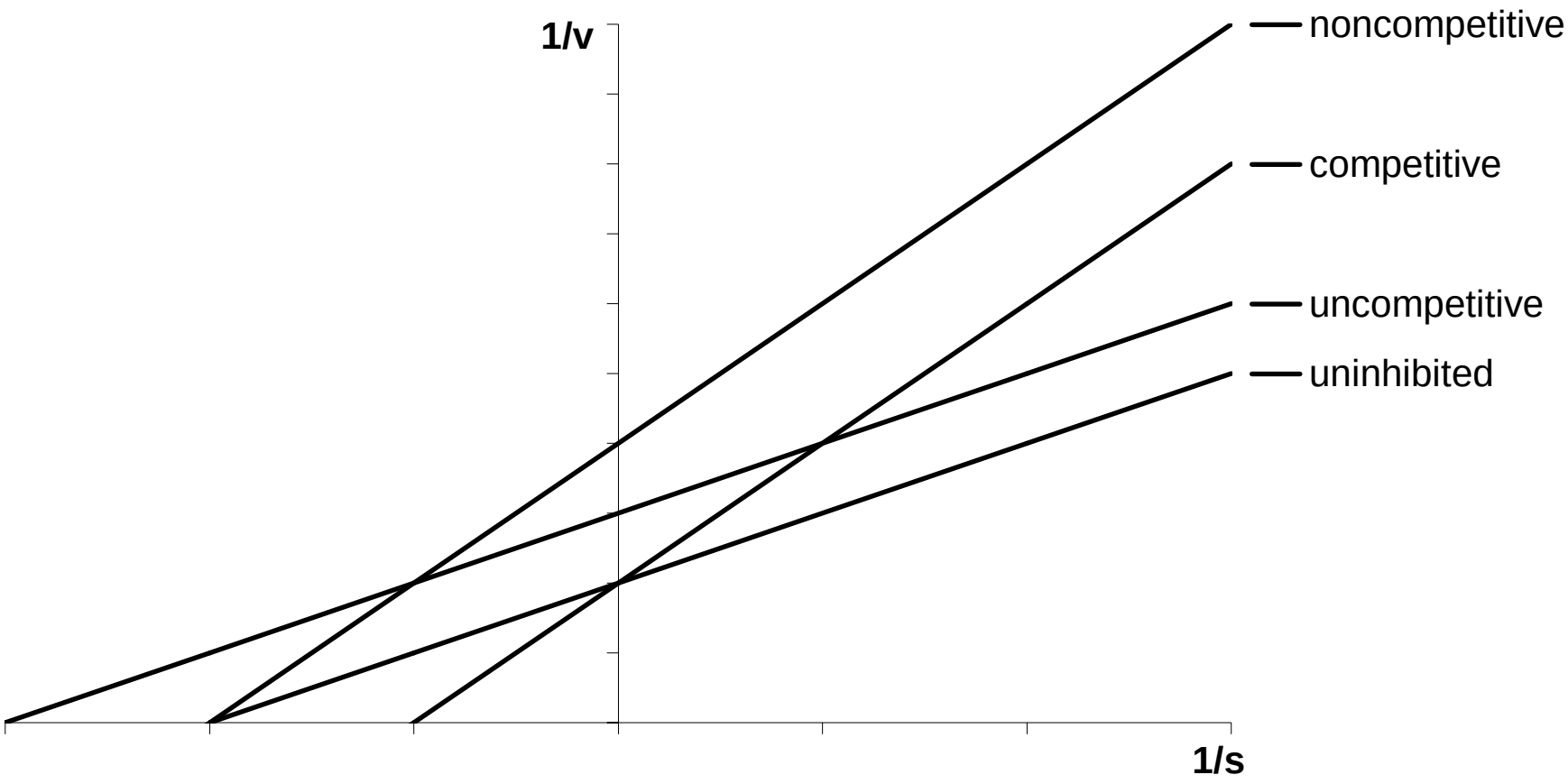
2. Uncompetitive inhibition

- **a.** Inhibitors bind only to the ES complex at a site distinct from the active site (i.e., the allosteric site).
- **b.** For an uncompetitive inhibitor, K_i is defined as the dissociation constant for the ES-inhibitor complex.

3. Noncompetitive inhibition

- **a.** Inhibitors bind both to the free enzyme and to the ES at the allosteric site, which is distinct from the active site.
- **b. For a "pure" noncompetitive inhibitor,** the dissociation constants for the binding of the inhibitor to the enzyme and ES are identical.
- **c. For a "mixed" noncompetitive inhibitor,** the dissociation constants for the binding of the inhibitor to the enzyme and ES are not equal.

Lineweaver-Burk plots showing the effects of uncompetitive, competitive, and noncompetitive inhibitors on the kinetics of enzyme-catalyzed reactions.



<u>Irreversible competitive inhibitors</u> bind covalently or so tightly to the active site that the enzyme is inactivated irreversibly. Irreversible inhibition does not obey michaelis-menten kinetics.

1. **Affinity labels**
   a. **Definition.** These are substrate analogs that possess a highly reactive group that is not present on the natural substrate.
   b. **Action.** The active site is permanently blocked from the substrate because the group reacts covalently with an amino acid residue. The residue that is modified is not necessarily involved in catalysis.
2. **Mechanism-based or suicide inhibitors**
   a. **Definition.** These are substrate analogs that are transformed by the catalytic action of the enzyme.
   b. **Action.** Their structures are such that the product of this reaction is highly reactive and subsequently combines covalently with an amino acid residue in the active site, thus inactivating the enzyme.
3. **Transition-state analogs**
   a. **Definition.** These are substrate analogs whose structures closely resemble the transition state of the natural substrate.
   b. **Action.** Transition-state analogs do not covalently modify the enzyme but bind the active site so tightly that they irreversibly inactivate it.

MULTIPLE FACTORS AFFECT THE RATES OF ENZYME-CATALYSED REACTIONS

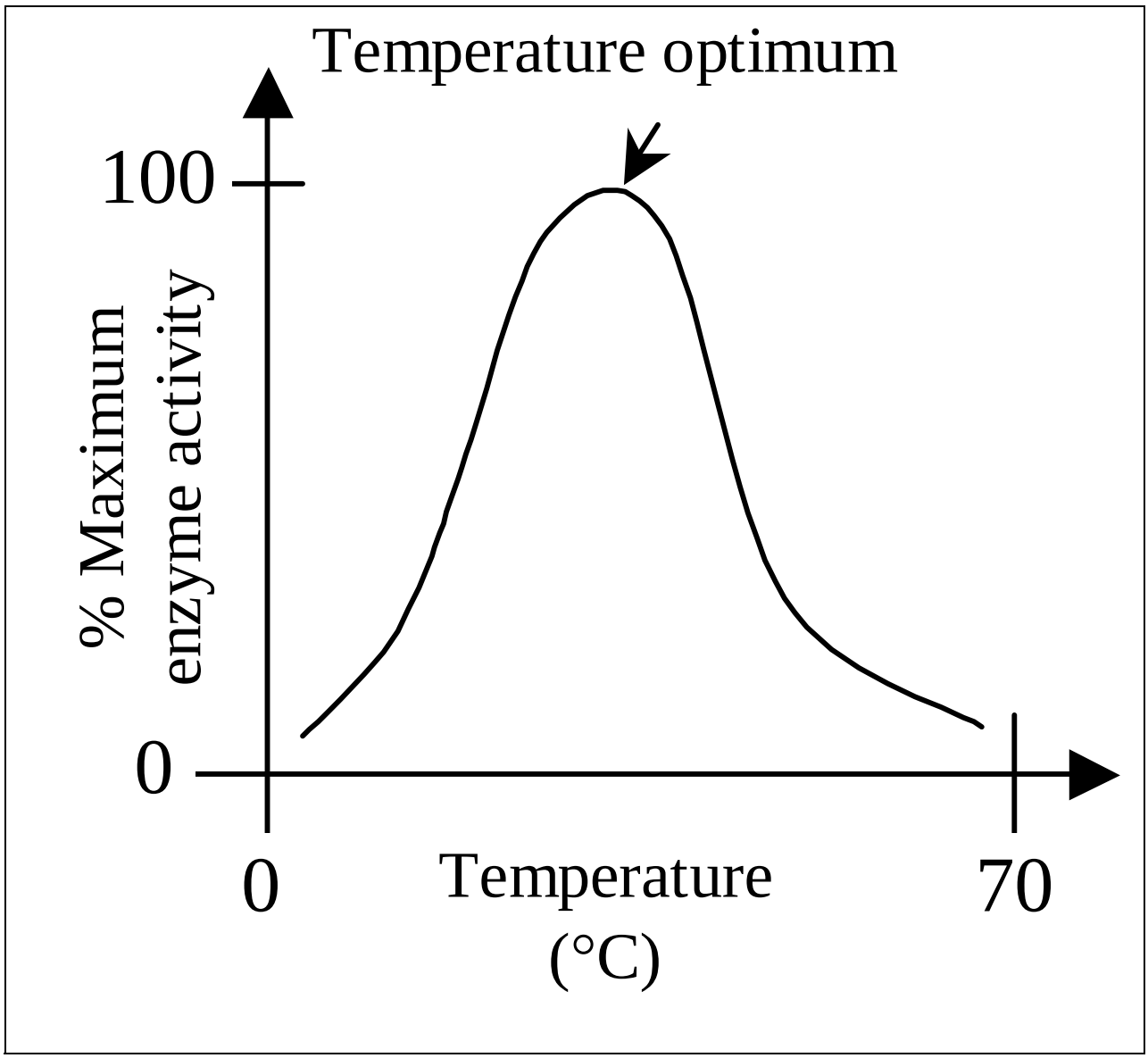
pH. A change in pH can alter the rates of enzyme-catalyzed reactions, with many enzymes exhibiting a bell-shaped curve when enzyme activity is plotted against pH. Changes in pH can alter the following:

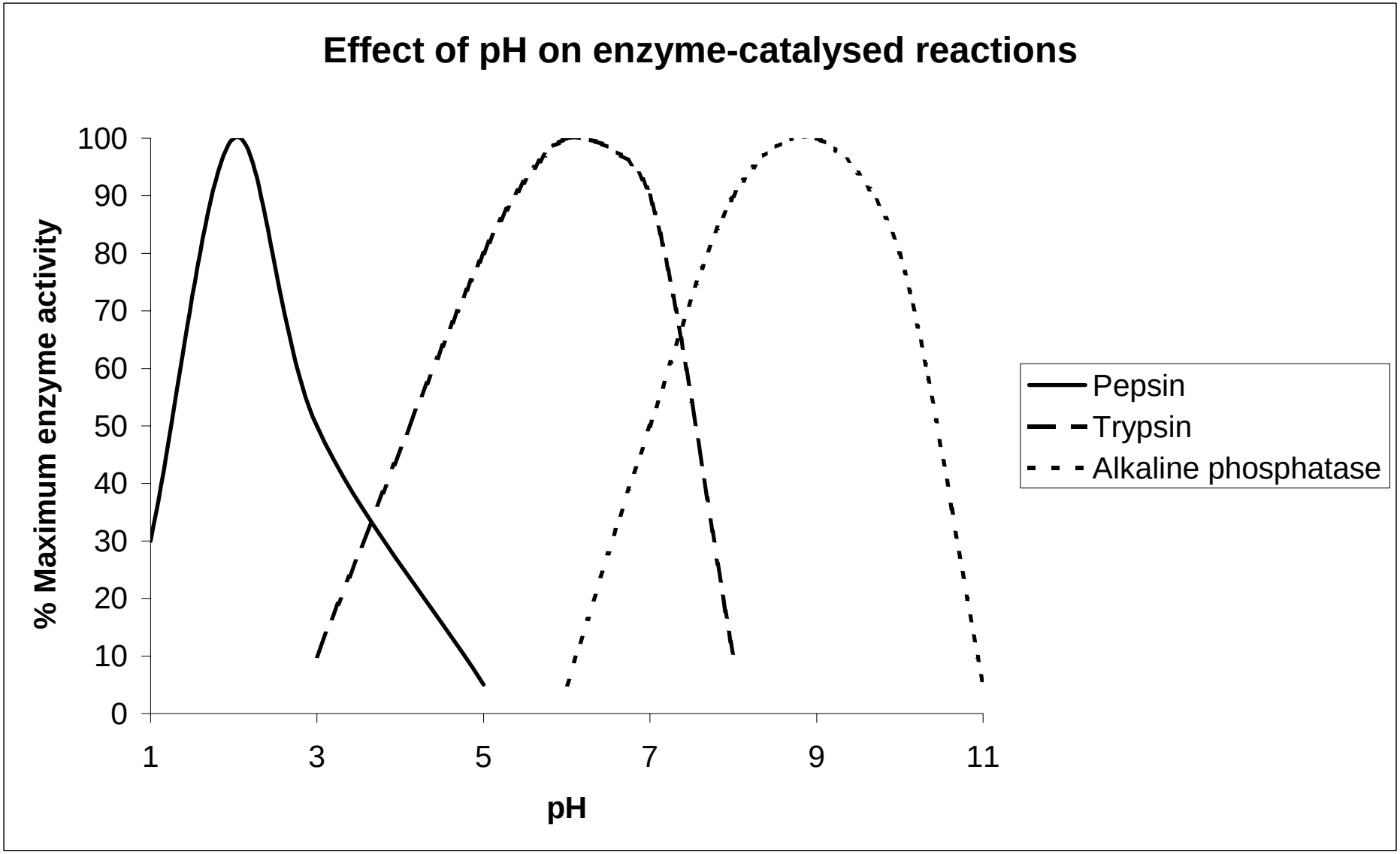
1. **The ionization state** of the substrate or the enzyme-binding site for substrate
2. The ionization state at the catalytic site on the enzyme
3. **Protein molecules** so that their conformation and catalytic activity change

Temperature. The rate of an enzyme-catalyzed reaction usually increases with increasing temperature up to an **optimum point**, then it decreases because enzymes are **thermolabile.**

Enzymes do not affect equilibrium constants

Effect of temperature on velocity of a hypothetical enzyme-catalyzed reaction


Temperature optimum
100
% Maximum enzyme activity
0
0
Temperature (°C)
70


Effect of pH on enzyme-catalysed reactions
% Maximum enzyme activity
100
90
80
70
60
50
40
30
20
10
0
1
3
5
7
9
11
pH
Pepsin
Trypsin
Alkaline phosphatase

Examples of Enzyme Inhibitors with Therapeutic Applications

Inhibitor	Target Enzyme	Effect or Application
Allopurinol	**Xanthine oxidase**	**Treatment of gout**
Aspirin	**Cyclooxygenase**	**Antiinflammatory agent**
5-Fluorouracil	**Thymidylate synthetase**	**Antineoplastic agent**
Lovastatin	**HMG-CoA reductase**	**Cholesterol-lowering agent**
Pargyline	**Monoamine oxidase**	**Antihypertensive agent**
Penicillin	**Transpeptidase**	**Antibacterial agent**

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A.