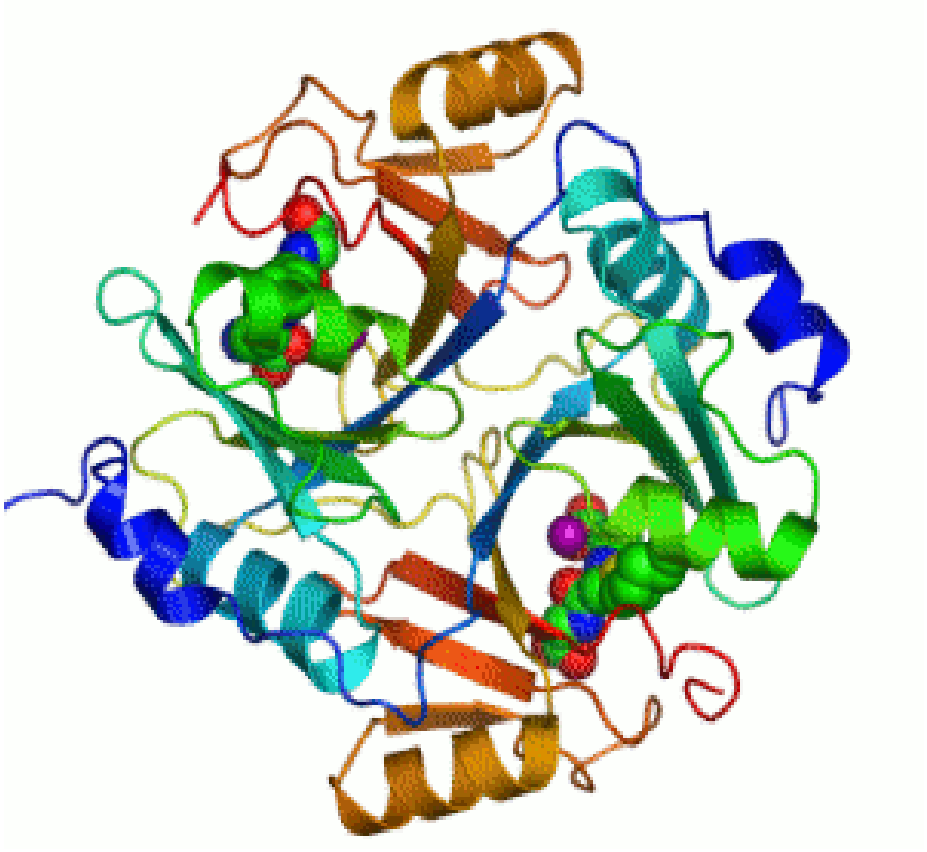
AL-RASHEED UNIVERSITY COLLEGE
DEPARTMENT OF MEDICAL LABORATORY TECHNIQUES

Enzymes

Lecture 3



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الوحدة الأولى - المحاضرة الثالثة - الزمن: 90 دقيقة

أهداف المحاضرة الثالثة:

يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:

By the end of the lecture, the student should be able to:

1. Define the following terms: (First-order and zero-order kinetics, K_m, V_{max}, Enzyme inhibition (competitive, noncompetitive, uncompetitive)
2. State the Michaelis-Menten and Lineweaver-Burk equations and relate them to enzyme kinetics by defining reaction velocity, V_{max}, and K_m.
3. Draw and label a Michaelis-Menten curve and a Lineweaver-Burk plot.
4. State the way in which each type of inhibition affects enzyme kinetics and illustrate how each of the three types affects the enzymatic reaction rate using a Lineweaver-Burk plot.

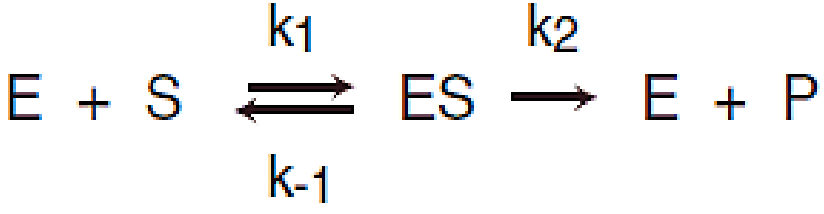
موضوعات المحاضرة الثالثة:

- MICHAELIS-MENTEN EQUATION
  - Reaction model
  - Characteristics of Km:
  - Relationship of velocity to enzyme concentration:
  - Order of reaction:
  - Lineweaver-Burk plot
- INHIBITION OF ENZYME ACTIVITY
  - Competitive Inhibition
  - Noncompetitive inhibition
  - Enzyme inhibitors as drugs

VI. MICHAELIS-MENTEN EQUATION

A. Reaction model

- Leonor Michaelis and Maude Menten proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.
- In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme. The model, involving one substrate molecule, is represented below:



where S is the substrate
E is the enzyme
ES is the enzyme–substrate complex
P is the product
k_1, k_{-1}, and k_2 are rate constants

B. Michaelis-Menten equation

- The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

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

where v_o = initial reaction velocity

V_{max} = maximal velocity

K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$

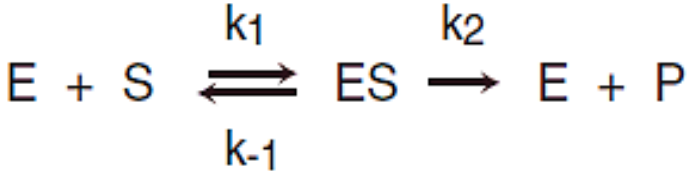
[S] = substrate concentration

The following assumptions are made in deriving the Michaelis-Menten rate equation:

1. **Relative concentrations of E and S:**
   - The concentration of substrate ([S]) is much greater than the concentration of enzyme ([E]), so that the percentage of total substrate bound by the enzyme at any one time is small.

2. Steady-state assumption:

- [ES] does not change with time (the steady-state assumption), that is, the rate of formation of ES is equal to that of the breakdown of ES (to E + S and to E + P).



- In general, an intermediate in a series of reactions is said to be in steadystate when its rate of synthesis is equal to its rate of degradation.

3. Initial velocity:

- Initial reaction velocities (v_o) are used in the analysis of enzyme reactions. This means that the rate of the reaction is measured as soon as enzyme and substrate are mixed. At that time, the concentration of product is very small and, therefore, the rate of the back reaction from P to S can be ignored.

C. Important conclusions

1. Characteristics of K_m:

- K_m, the Michaelis constant, is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate.
- K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to $\frac{1}{2}V_{max}$.
- K_m does not vary with the concentration of enzyme.

a. Small K_m: A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is $\frac{1}{2}V_{max.}$ (Figure 1.8).

b. Large K_m: A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

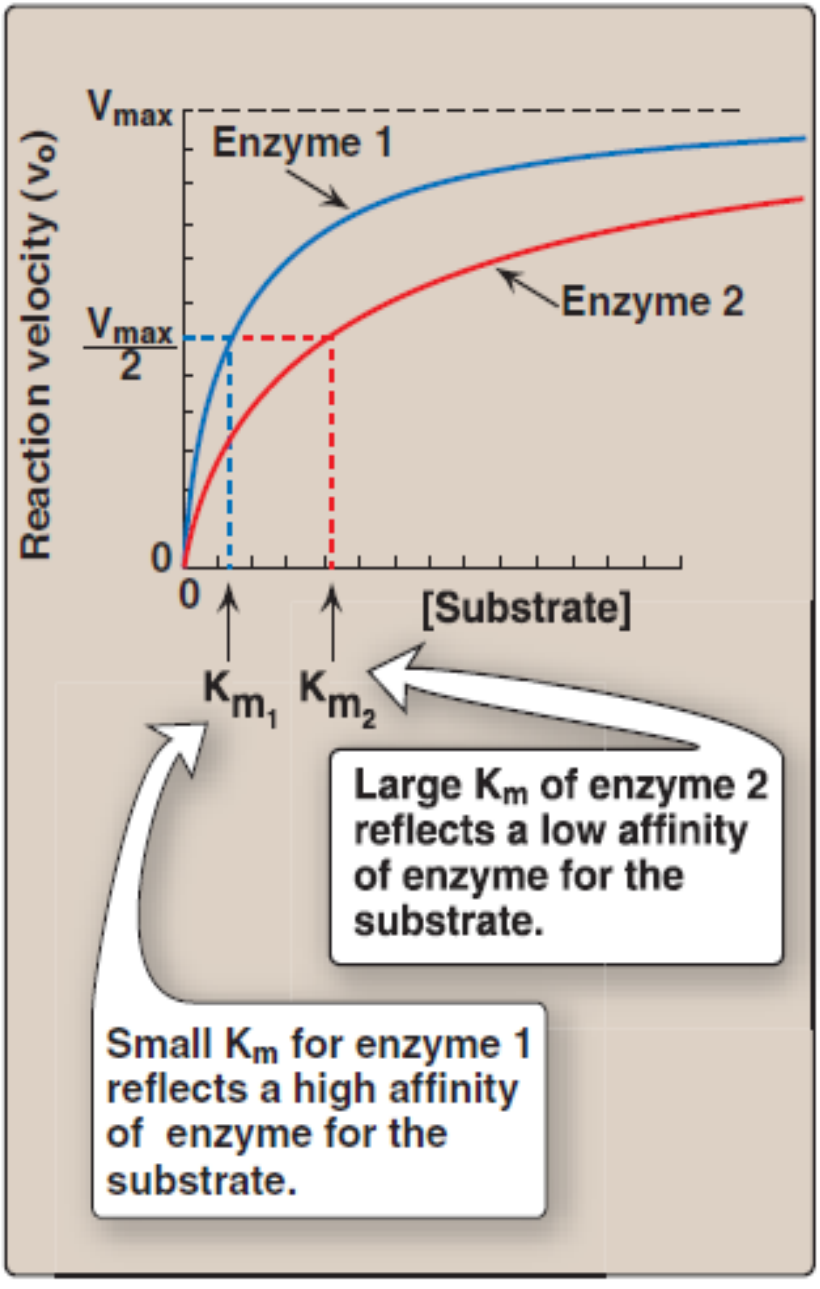


Figure 1.8 Effect of substrate concentration on reaction velocities for two enzymes: enzyme 1 with a small K_m, and enzyme 2 with a large K_m.

2. Relationship of velocity to enzyme concentration:

- The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations.
- For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as that of $V_{max,}$ are reduced to half that of the original.

3. Order of reaction:

- When [S] is much less than $K_{m,}$ the velocity of the reaction is approximately proportional to the substrate concentration (Figure 1.9). The rate of reaction is then said to be first order with respect to substrate.
- When [S] is much greater than K_m, the velocity is constant and equal to $V_{max.}$ The rate of reaction is then independent of substrate concentration (the enzyme is saturated with substrate) and is said to be zero order with respect to substrate concentration (see Figure 1.9).

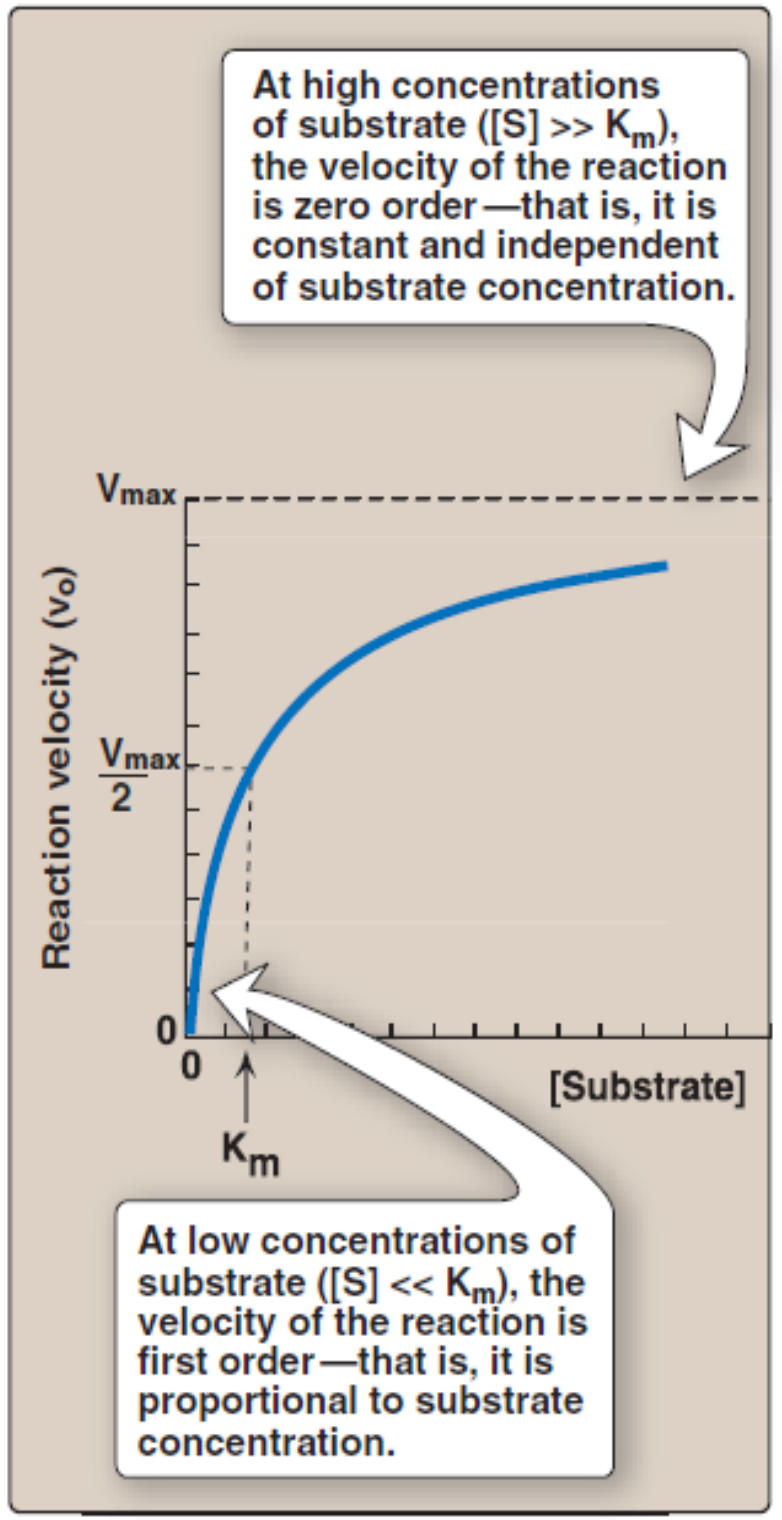


Figure 1.9 Effect of substrate concentration on reaction velocity for an enzyme-catalyzed reaction.

D. Lineweaver-Burk plot

- When v_o is plotted against [S], it is not always possible to determine when V_{max} has been achieved, because of the gradual upward slope of the hyperbolic curve at high substrate concentrations.
- However, if $1/v_o$ is plotted versus $1/[S]$, a straight line is obtained (Figure 1.10).
- This plot, the Lineweaver-Burk plot (also called a double-reciprocal plot) can be used to calculate K_m and V_{max}, as well as to determine the mechanism of action of enzyme inhibitors.

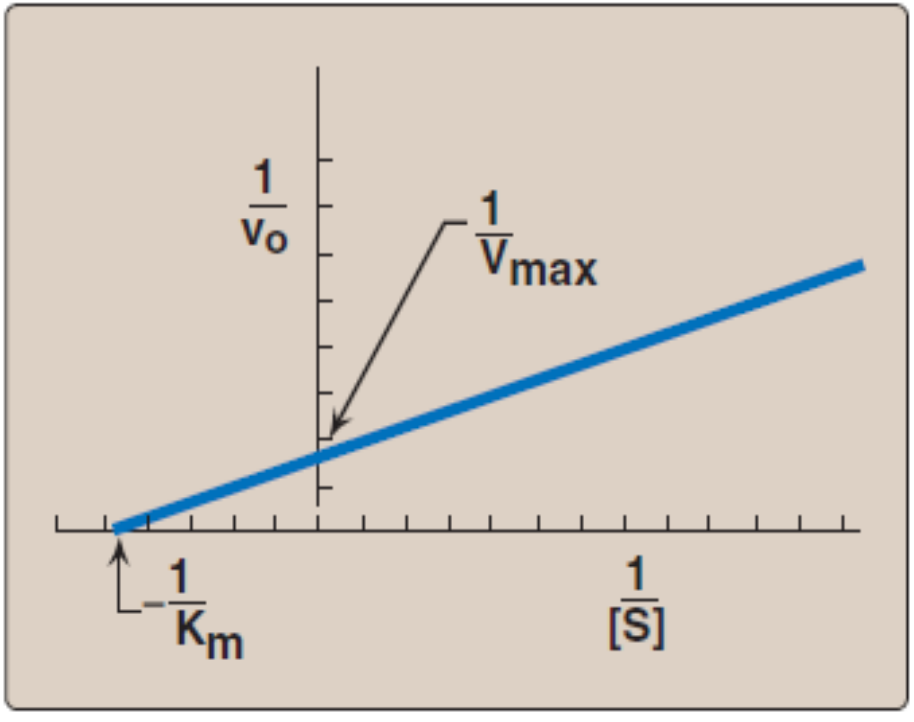


Figure 1.10 Lineweaver-Burk plot.

- The equation describing the Lineweaver-Burk plot is:

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

where the intercept on the x-axis is equal to $-1/K_m$, and the intercept on the y-axis is equal to $1/V_{max}$.

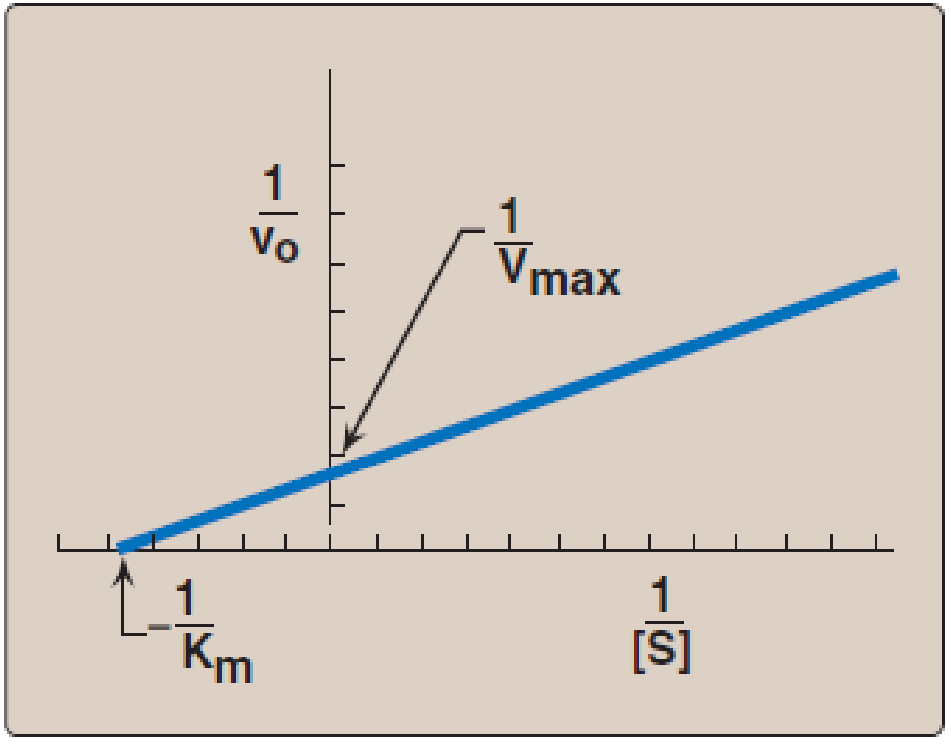


Figure 1.10 Lineweaver-Burk plot.

VII. INHIBITION OF ENZYME ACTIVITY

- Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor.
- Inhibitors can be reversible or irreversible.
- Irreversible inhibitors bind to enzymes through covalent bonds.
- Reversible inhibitors typically bind to enzymes through noncovalent bonds, thus dilution of the enzyme–inhibitor complex results in dissociation of the reversibly bound inhibitor, and recovery of enzyme activity.
- The two most commonly encountered types of reversible inhibition are competitive and noncompetitive.

A. Competitive Inhibition

- This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and, therefore, competes with the substrate for that site.

1. **Effect on V_{max}:** The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor (Figure 1.11).

2. **Effect on K_m:** A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve ½V_{max}.

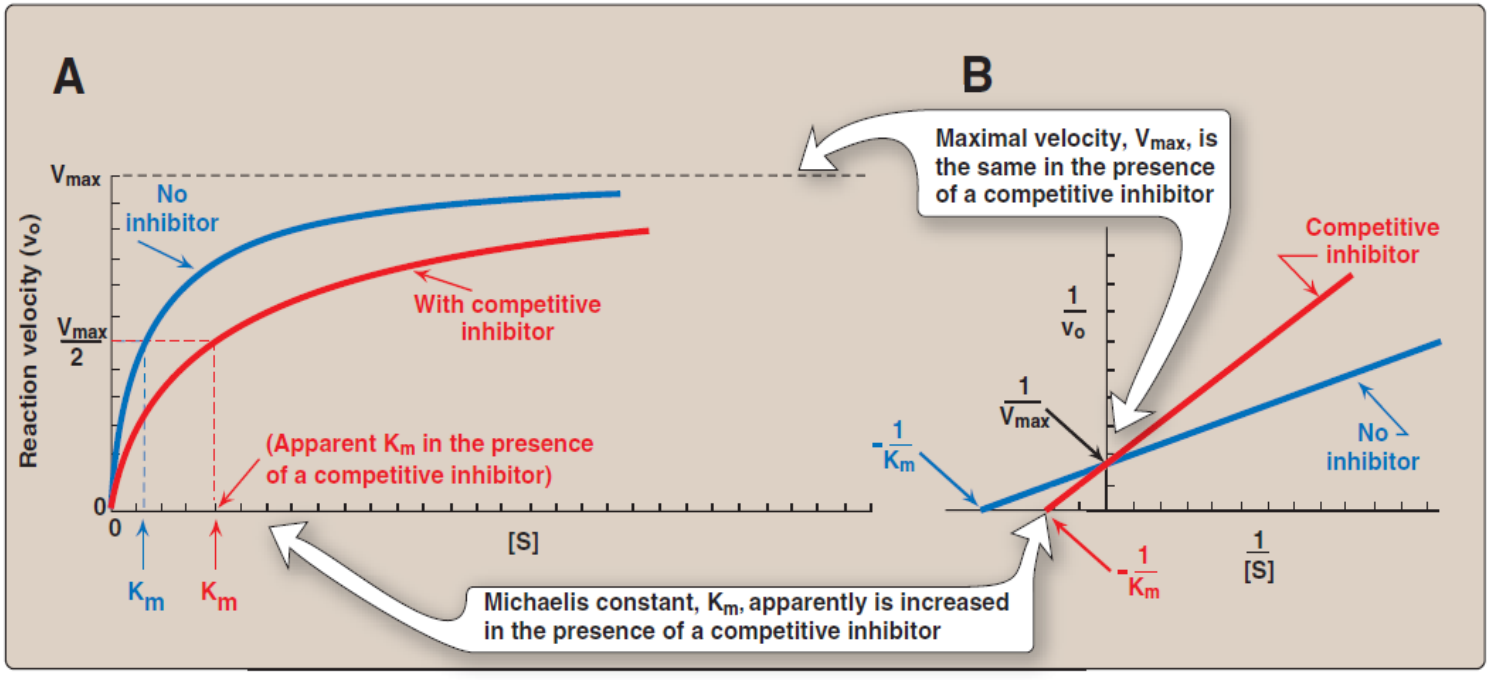


Figure 1.11 A. Effect of a competitive inhibitor on the reaction velocity (v_o) versus substrate ([S]) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.

3. Effect on the Lineweaver-Burk plot:

- Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited and uninhibited reactions intersect on the y-axis at $1/V_{max}$ (V_{max} is unchanged).
- The inhibited and uninhibited reactions show different x-axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor because $-1/K_m$ moves closer to zero from a negative value (see Figure 1.11).

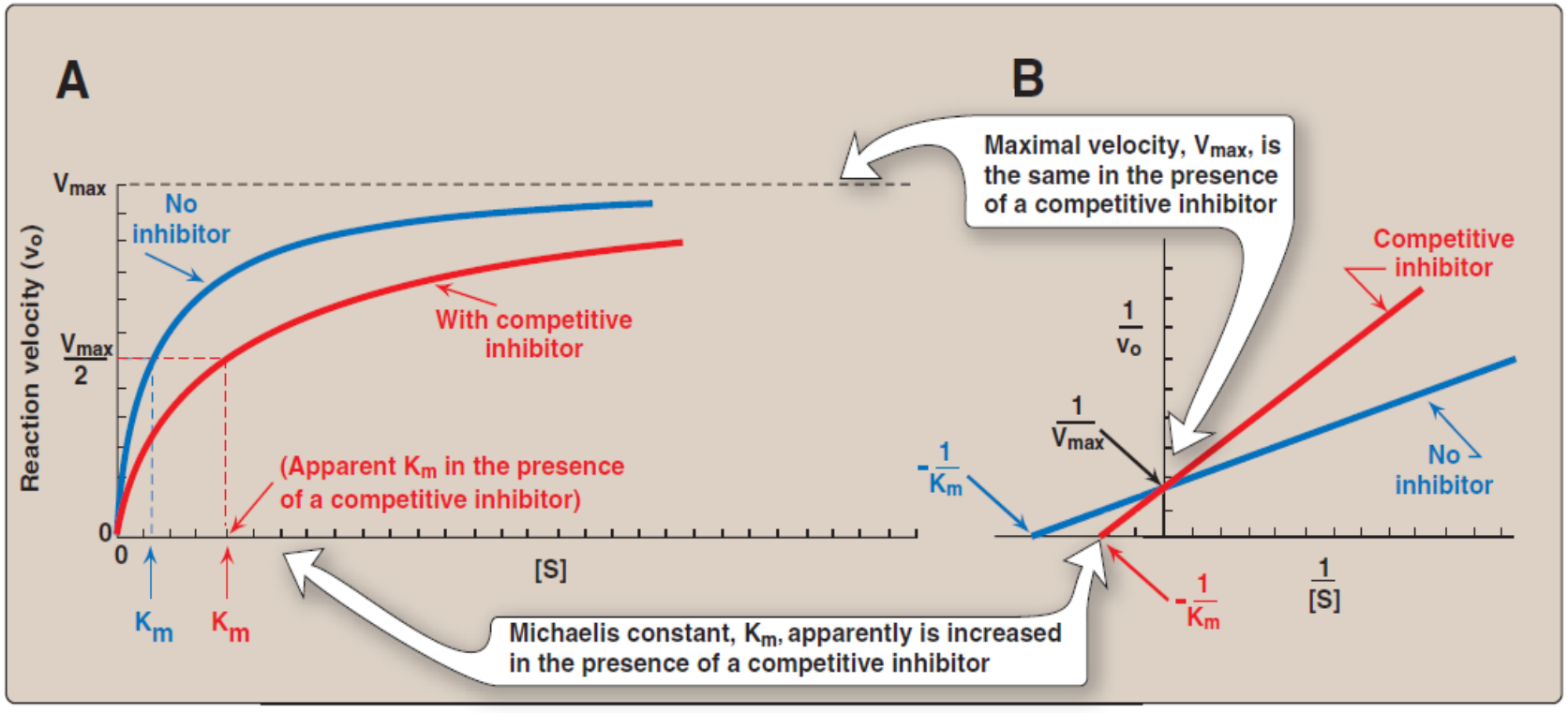


Figure 1.11 A. Effect of a competitive inhibitor on the reaction velocity (v_o) versus substrate ([S]) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.

4. Statin drugs as examples of competitive inhibitors

- This group of antihyperlipidemic agents competitively inhibits the rate-limiting (slowest) step in cholesterol biosynthesis

- This reaction is catalyzed by *hydroxymethylglutaryl–CoA reductase* (*HMG-CoA reductase*).

- Statin drugs, such as atorvastatin (Lipitor) and pravastatin (Pravachol), are structural analogs of the natural substrate for this enzyme, and compete effectively to inhibit *HMG-CoA reductase*. By doing so, they inhibit de novo cholesterol synthesis, thereby lowering plasma cholesterol levels (Figure 1.12).

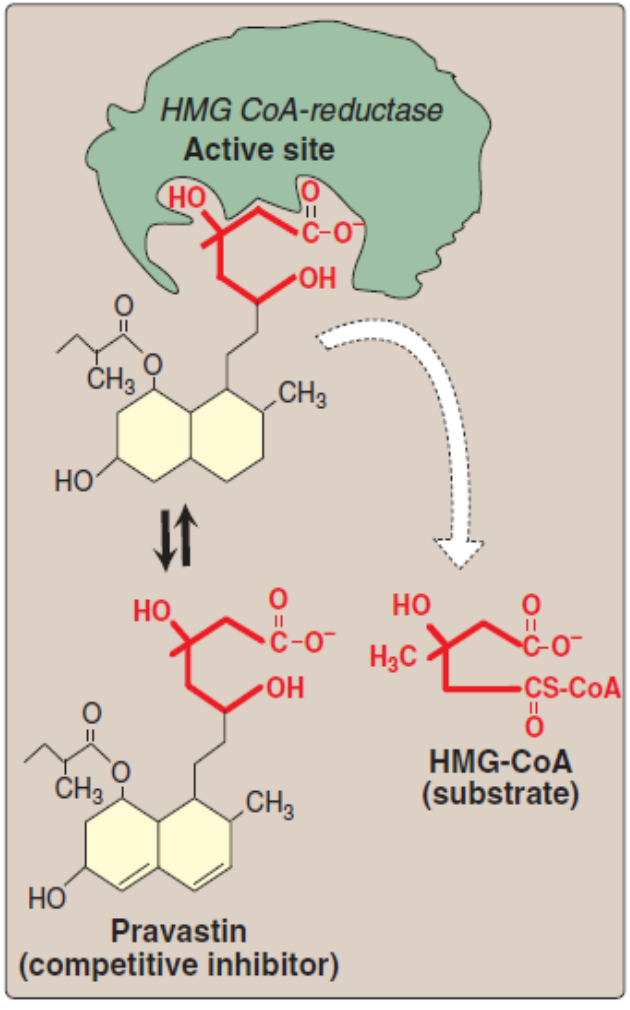


Figure 1.12 Pravastatin competes with HMG-CoA or the active site of *HMG-CoA reductase*.

B. Noncompetitive inhibition

- This type of inhibition is recognized by its characteristic effect on V_{max} (Figure 1.13).
- Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme.
- The noncompetitive inhibitor can bind either free enzyme or the ES complex, thereby preventing the reaction from occurring (Figure 1.14).

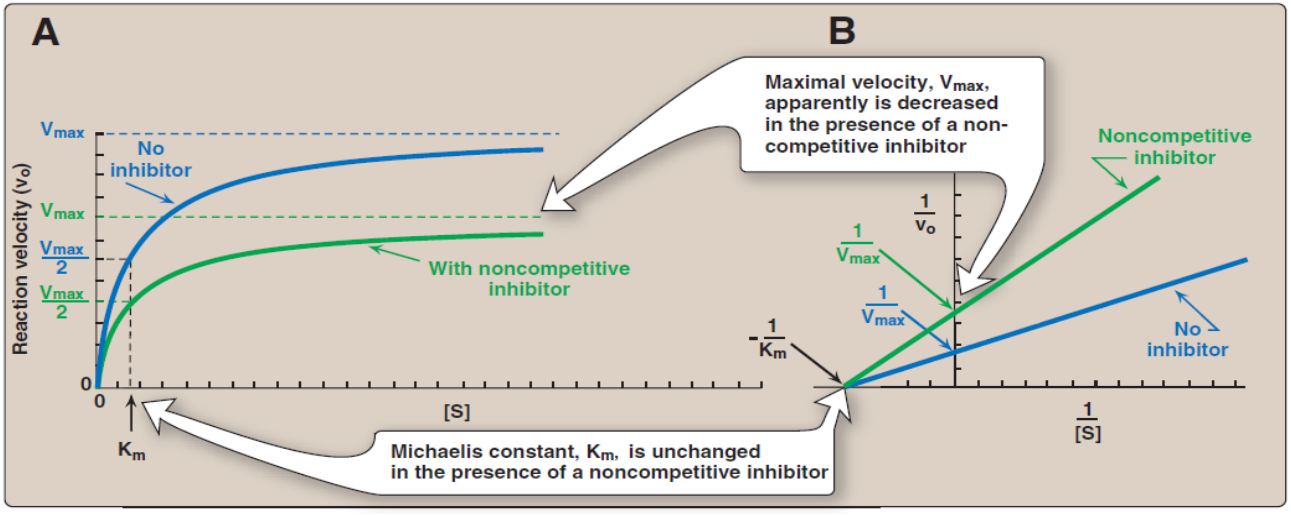


Figure 1.13 A. Effect of a noncompetitive inhibitor on the reaction velocity (vo) versus substrate ([S]) plot. B. Lineweaver-Burk plot of noncompetitive inhibition of an enzyme.

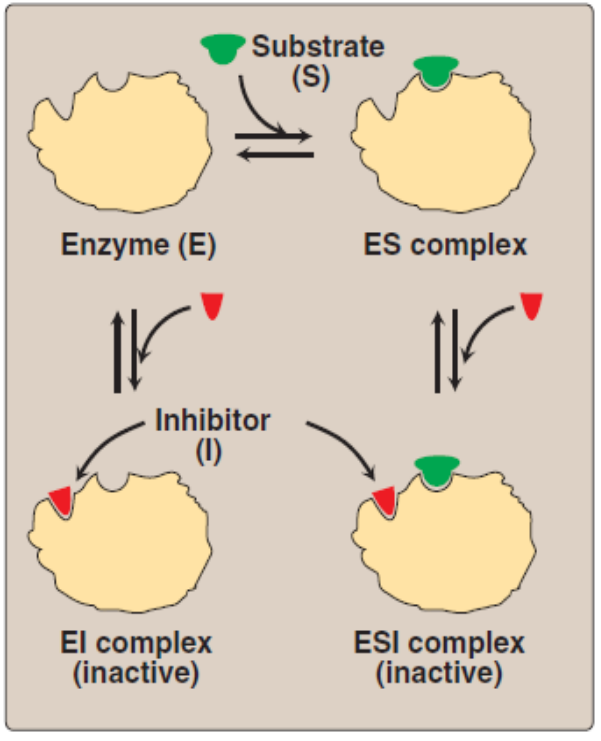
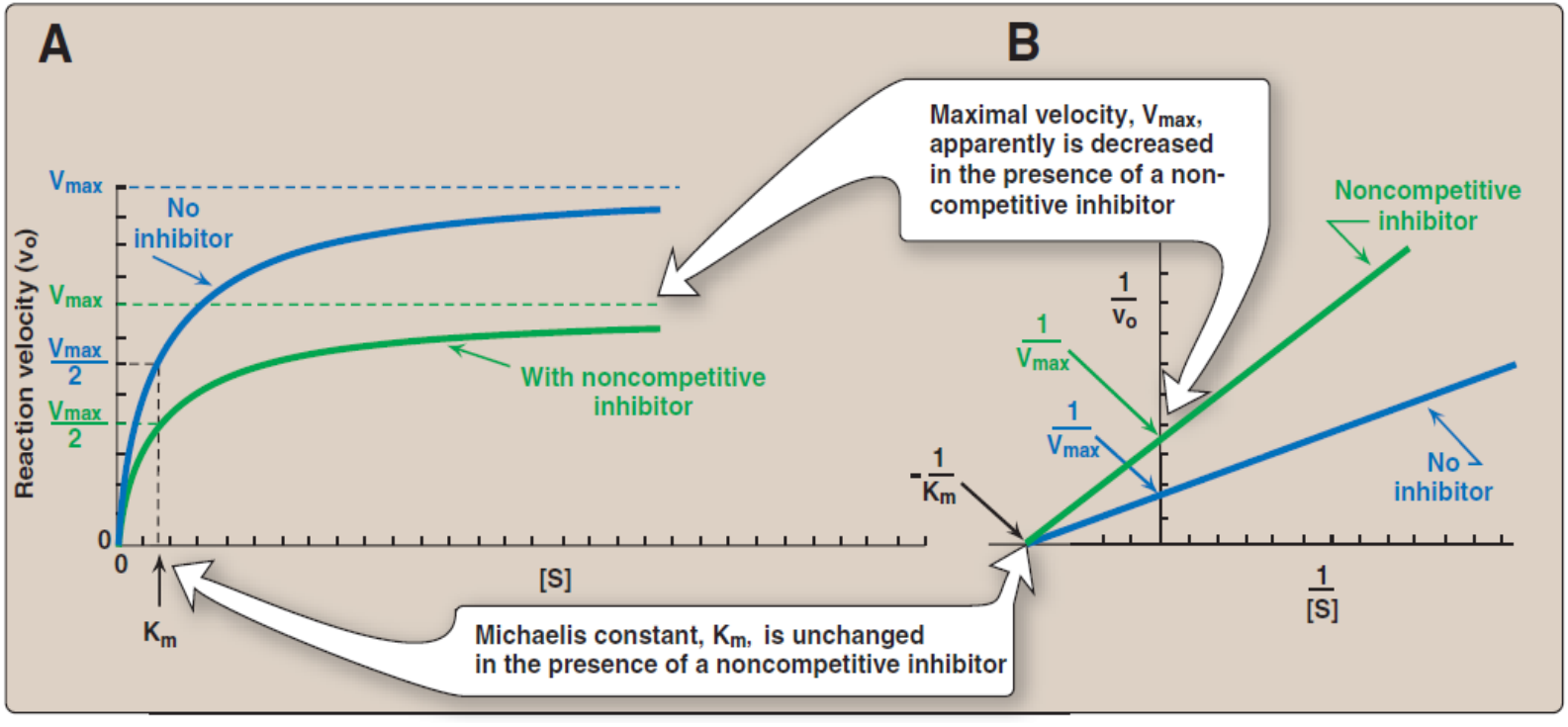


Figure 1.14 A noncompetitive inhibitor binding to both free enzyme and enzyme–substrate (ES) complex.

1. **Effect on V_{max}:** Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, noncompetitive inhibitors decrease the apparent V_{max} of the reaction.

2. **Effect on K_m:** Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Therefore, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

3. **Effect on Lineweaver-Burk plot:** Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting $1/v_o$ versus $1/[S]$ and noting that the apparent V_{max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged (see Figure 1.13).



4. Examples of noncompetitive inhibitors:

- Some inhibitors act by forming covalent bonds with specific groups of enzymes.
- For example, lead forms covalent bonds with the sulfhydryl side chains of cysteine in proteins. The binding of the heavy metal shows noncompetitive inhibition.
- *Ferrochelatase*, an enzyme that catalyzes the insertion of Fe^{2+} into protoporphyrin (a precursor of heme), is an example of an enzyme sensitive to inhibition by lead .

Why are heavy metals often poisons in the body?

- Examples are the reaction of the heavy metals mercury, silver, and lead with sulfhydryl groups (–SH) on the enzyme. The sulfhydryl group is tied up by the heavy metal ($ESH + Ag^{+} \rightarrow ESAg + H^{+}$), and this reaction is irreversible. This is why heavy metals are poisons; they inactivate enzymes in the body.

Competitive Inhibition	Noncompetitive Inhibition
➢ Competitive inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy	➢ Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme and can bind either free enzyme or the ES complex,
➢ V_{max}, is the same	➢ V_{max} is decreased
➢ K_m, is increased	➢ K_m, is unchanged
➢ Statin drugs as examples of competitive inhibitors. Statin drugs, such as pravastatin, are structural analogs of the natural substrate for this enzyme, and compete effectively to inhibit *HMG-CoA reductase*.	➢ For example, lead forms covalent bonds with the sulfhydryl side chains of cysteine in proteins. The binding of the heavy metal shows noncompetitive inhibition.

C. Enzyme inhibitors as drugs

- At least half of the ten most commonly dispensed drugs act as enzyme inhibitors.
- For example, the widely prescribed β-lactam antibiotics, such as penicillin and amoxicillin, act by inhibiting enzymes involved in bacterial cell wall synthesis.

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Answer the Following Question

State the Michaelis-Menten and Lineweaver-Burk equations and relate them to enzyme kinetics by defining reaction velocity, V_{max}, and K_m.

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Compare between Competitive & Noncompetitive inhibitions