1. Keeping the Sweet Taste of Corn  The sweet taste of freshly picked corn (maize) is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet, because about 50% of the free sugar is converted to starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water. Corn processed in this way and stored in a freezer maintains its sweetness. What is the biochemical basis for this procedure?

**Answer**  After an ear of corn has been removed from the plant, the enzyme-catalyzed conversion of sugar to starch continues. Inactivation of these enzymes slows down the conversion to an imperceptible rate. One of the simplest techniques for inactivating enzymes is heat denaturation. Freezing the corn lowers any remaining enzyme activity to an insignificant level.

2. Intracellular Concentration of Enzymes  To approximate the actual concentration of enzymes in a bacterial cell, assume that the cell contains equal concentrations of 1,000 different enzymes in solution in the cytosol and that each protein has a molecular weight of 100,000. Assume also that the bacterial cell is a cylinder (diameter 1.0 \( \mu \text{m} \), height 2.0 \( \mu \text{m} \)), that the cytosol (specific gravity 1.20) is 20% soluble protein by weight, and that the soluble protein consists entirely of enzymes. Calculate the average molar concentration of each enzyme in this hypothetical cell.

**Answer**  There are three different ways to approach this problem.

(i) The concentration of total protein in the cytosol is

\[
\frac{(1.2 \text{ g/mL})(0.20)}{100,000 \text{ g/mol}} = 0.24 \times 10^{-5} \text{ mol/mL} = 2.4 \times 10^{-3} \text{ M}
\]

Thus, for 1 enzyme in 1,000, the enzyme concentration is

\[
\frac{2.4 \times 10^{-3} \text{ M}}{1000} = 2.4 \times 10^{-6} \text{ M}
\]

(ii) The average molar concentration = \( \frac{\text{moles of each enzyme in cell}}{\text{volume of cell in liters}} \)

Volume of bacterial cytosol \( = \pi r^2 h = (3.14)(0.50 \mu \text{m})^2(2.0 \mu \text{m}) = 1.6 \mu \text{m}^3 \)

\( = 1.6 \times 10^{-12} \text{ cm}^3 = 1.6 \times 10^{-12} \text{ mL} = 1.6 \times 10^{-15} \text{ L} \)

Amount (in moles) of each enzyme in cell is

\[
\frac{(0.20)(1.2 \text{ g/cm}^3)(1.6 \mu \text{m}^3)(10^{-12} \text{ cm}^3/\mu \text{m}^3)}{100,000 \text{ g/mol}(1000)} = 3.8 \times 10^{-21} \text{ mol}
\]

Average molar concentration \( = \frac{3.8 \times 10^{-21} \text{ mol}}{1.6 \times 10^{-15} \text{ L}} \)

\( = 2.4 \times 10^{-6} \text{ mol/L} = 2.4 \times 10^{-6} \text{ M} \)
(iii) Volume of bacterial cytosol

\[ \text{Volume} = \pi r^2 h = (3.14)(0.50 \, \mu\text{m})^2(2.0 \, \mu\text{m}) = 1.6 \, \mu\text{m}^3 = 1.6 \times 10^{-12} \text{ mL} \]

Weight of cytosol = (specific gravity)(volume)

\[ \text{Weight} = (1.2 \, \text{g/mL})(1.6 \times 10^{-12} \, \text{mL}) = 1.9 \times 10^{-12} \, \text{g} \]

Average weight of each protein (1 in 1,000, 20% wt/wt protein)

\[ \text{Average weight} = (1.9 \times 10^{-12} \, \text{g})(0.20)/(1,000) = 3.8 \times 10^{-16} \, \text{g} \]

Average molar concentration of each protein

\[ \text{Average molar concentration} = (3.8 \times 10^{-16} \, \text{g})/(10^5 \, \text{g/mol})(1.6 \times 10^{-12} \, \text{mL})(1 \, \text{L}/1000 \, \text{mL}) = 2.4 \times 10^{-6} \, \text{mol/L} = 2.4 \times 10^{-6} \, \text{M} \]

3. Rate Enhancement by Urease

The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20 °C by a factor of \(10^{14}\). If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20 °C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

**Answer**

Time to hydrolyze urea

\[ \text{Time} = \frac{(5.0 \, \text{min})(10^{14})}{(60 \, \text{min/hr})(24 \, \text{hr/day})(365 \, \text{days/yr})} = 9.5 \times 10^8 \, \text{yr} = 950 \, \text{million years!} \]

4. Protection of an Enzyme against Denaturation by Heat

When enzyme solutions are heated, there is a progressive loss of catalytic activity over time due to denaturation of the enzyme. A solution of the enzyme hexokinase incubated at 45 °C lost 50% of its activity in 12 min, but when incubated at 45 °C in the presence of a very large concentration of one of its substrates, it lost only 3% of its activity in 12 min. Suggest why thermal denaturation of hexokinase was retarded in the presence of one of its substrates.

**Answer**

One possibility is that the ES complex is more stable than the free enzyme. This implies that the ground state for the ES complex is at a lower energy level than that for the free enzyme, thus increasing the height of the energy barrier to be crossed in passing from the native to the denatured or unfolded state.

An alternative view is that an enzyme denatures in two stages: reversible conversion of active native enzyme (N) to an inactive unfolded state (U), followed by irreversible conversion to inactivated enzyme (I):

\[ N \rightleftharpoons U \rightarrow I \]

If substrate, S, binds only to N, saturation with S to form NS would leave less free N available for conversion to U or I, as the N \rightleftharpoons U equilibrium is perturbed toward N. If N but not NS is converted to U or I, then substrate binding will cause stabilization.

5. Requirements of Active Sites in Enzymes

Carboxypeptidase, which sequentially removes carboxyl-terminal amino acid residues from its peptide substrates, is a single polypeptide of 307 amino acids. The two essential catalytic groups in the active site are furnished by Arg\(^{145}\) and Gln\(^{270}\).

(a) If the carboxypeptidase chain were a perfect \(\alpha\) helix, how far apart (in Å) would Arg\(^{145}\) and Gln\(^{270}\) be? (Hint: see Fig. 4–4a.)

(b) Explain how the two amino acid residues can catalyze a reaction occurring in the space of a few angstroms.
Answer
(a) Arg<sup>145</sup> is separated from Glu<sup>270</sup> by 270 – 145 = 125 amino acid (AA) residues. From Figure 4-4a we see that the α helix has 3.6 AA/turn and increases in length along the major axis by 5.4 Å/turn. Thus, the distance between the two residues is
\[
\frac{(125 \text{ AA})(5.4 \text{ Å/turn})}{3.6 \text{ AA/turn}} = 190 \text{ Å}
\]
(b) Three-dimensional folding of the enzyme brings the two amino acid residues into close proximity.

6. Quantitative Assay for Lactate Dehydrogenase The muscle enzyme lactate dehydrogenase catalyzes the reaction

\[
\text{CH}_3\text{C}-\text{COO}^- + \text{NADH} + \text{H}^+ \rightarrow \text{CH}_3\text{C}-\text{COO}^- + \text{NAD}^+
\]

NADH and NAD<sup>+</sup> are the reduced and oxidized forms, respectively, of the coenzyme NAD. Solutions of NADH, but not NAD<sup>+</sup>, absorb light at 340 nm. This property is used to determine the concentration of NADH in solution by measuring spectrophotometrically the amount of light absorbed at 340 nm by the solution. Explain how these properties of NADH can be used to design a quantitative assay for lactate dehydrogenase.

Answer The reaction rate can be measured by following the decrease in absorption at 340 nm (as NADH is converted to NAD<sup>+</sup>) as the reaction proceeds. The researcher needs to obtain three pieces of information to develop a good quantitative assay for lactate dehydrogenase:
(i) Determine \( K_m \) values (see Box 6-1).
(ii) Measure the initial rate at several known concentrations of enzyme with saturating concentrations of NADH and pyruvate.
(iii) Plot the initial rates as a function of [E]; the plot should be linear, with a slope that provides a measure of lactate dehydrogenase concentration.

7. Effect of Enzymes on Reactions Which of the following effects would be brought about by any enzyme catalyzing the simple reaction

\[
S \xrightarrow{k_1 \quad k_2} P \quad \text{where} \quad K_{eq}^* = \frac{[P]}{[S]}
\]

(a) Decreased \( K_{eq}^* \); (b) Increased \( k_1 \); (c) Increased \( K_{eq}^* \); (d) Increased \( \Delta G^* \); (e) Decreased \( \Delta G^* \); (f) More negative \( \Delta G^* \); (g) Increased \( k_2 \).

Answer (b), (e), (g). Enzymes do not change a reaction’s equilibrium constant and thus catalyze the reaction in both directions, making (b) and (g) correct. Enzymes increase the rate of a reaction by lowering the activation energy, hence (e) is correct.

8. Relation between Reaction Velocity and Substrate Concentration: Michaelis-Menten Equation
(a) At what substrate concentration would an enzyme with a \( k_{cat} \) of 30.0 s<sup>-1</sup> and a \( K_m \) of 0.0050 m operate at one-quarter of its maximum rate?
(b) Determine the fraction of \( V_{max} \) that would be obtained at the following substrate concentrations [S]: \( \frac{1}{2}K_m \), \( 2K_m \), and \( 10K_m \).
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(c) An enzyme that catalyzes the reaction X $\Rightarrow$ Y is isolated from two bacterial species. The enzymes have the same $V_{\text{max}}$, but different $K_m$ values for the substrate X. Enzyme A has a $K_m$ of 2.0 $\mu$M, while enzyme B has a $K_m$ of 0.5 $\mu$M. The plot below shows the kinetics of reactions carried out with the same concentration of each enzyme and with [X] = 1 $\mu$M. Which curve corresponds to which enzyme?

![Kinetics Plot]

Answer

(a) Here we want to find the value of [S] when $V_0 = 0.25 V_{\text{max}}$. The Michaelis-Menten equation is

$$V_0 = V_{\text{max}}[S]/(K_m + [S])$$

so $V_0 = V_{\text{max}}$ when $[S]/(K_m + [S]) = 0.25$; or

$$[S] = 0.33K_m = 0.33(0.0050 \text{ M}) = 1.7 \times 10^{-3} \text{ M}$$

(b) The Michaelis-Menten equation can be rearranged to

$$V_0/V_{\text{max}} = [S]/(K_m + [S])$$

Substituting $[S] = \frac{1}{2} K_m$ into the equation gives

$$V_0/V_{\text{max}} = 0.5 K_m/1.5K_m = 0.33$$

Similarly, substituting $[S] = 2K_m$ gives

$$V_0/V_{\text{max}} = 0.67$$

And substituting $[S] = 10K_m$ gives

$$V_0/V_{\text{max}} = 0.91$$

(c) The upper curve corresponds to enzyme B ([X] is greater than the $K_m$ for this enzyme), and the lower curve corresponds to enzyme A. When the initial concentration of substrate is greater than $K_m$, the rate of the reaction is less sensitive to the depletion of substrate at early stages of the reaction and the rate remains approximately linear for a longer time.

9. Applying the Michaelis-Menten Equation I  A research group discovers a new version of happyase, which they call happyase*, that catalyzes the chemical reaction

HAPPY $\longrightarrow$ SAD

The researchers begin to characterize the enzyme.

(a) In the first experiment, with [E] at 4 nM, they find that the $V_{\text{max}}$ is 1.6 $\mu$M s$^{-1}$. Based on this experiment, what is the $k_{\text{cat}}$ for happyase*? (Include appropriate units.)
(b) In another experiment, with [E$_t$] at 1 nm and [HAPPY] at 30 μM, the researchers find that $V_0$ = 300 nm s$^{-1}$. What is the measured $K_m$ of happyase* for its substrate HAPPY? (Include appropriate units.)

(c) Further research shows that the purified happyase* used in the first two experiments was actually contaminated with a reversible inhibitor called ANGER. When ANGER is carefully removed from the happyase* preparation, and the two experiments repeated, the measured $V_{max}$ in (a) is increased to 4.8 μM s$^{-1}$, and the measured $K_m$ in (b) is now 15 μM. For the inhibitor ANGER, calculate the values of $\alpha$ and $\alpha'$. 

(d) Based on the information given above, what type of inhibitor is ANGER?

**Answer**

(a) Use the equation $k_{cat} = V_{max}/[E_t]$. $k_{cat} = 1600$ nm s$^{-1}$/4 nm = 400 s$^{-1}$.

(b) Use the equation $V_{max} = k_{cat}[E_t]$. When [E$_t$] = 1 nm, $V_{max} = 400$ nm s$^{-1}$.

$$V_0/V_{max} = 300 \text{ nm s}^{-1}/400 \text{ nm s}^{-1} = \frac{3}{4}$$

Rearrange the Michaelis-Menten equation, substitute for $V_0/V_{max}$, and solve for $K_m$.

$$V_0/V_{max} = [S]/(K_m + [S])$$

$$\frac{3}{4} = [S]/(K_m + [S])$$

$$K_m = [S]/3$$

In this experiment, the concentration of the substrate, HAPPY, was 30 μM, so $K_m = 10$ μM.

(c) As shown in Table 6–9, $V_{max}$ varies as a function of $V_{max}/\alpha'$. Because $V_{max}$ increased by a factor of 3, $\alpha' = 3$. Similarly, $K_m$ varies as a function of $\alpha K_{cat}/\alpha'$. Given that $K_m$ increased by a factor of 1.5 when ANGER was removed (that is, the inhibitor decreased the observed $K_m$ by $\frac{2}{3}$) and $\alpha' = 3$, then $\alpha = 2$.

(d) Because both $\alpha$ and $\alpha'$ are affected, ANGER is a mixed inhibitor.

10. Applying the Michaelis-Menten Equation II Another enzyme is found that catalyzes the reaction

$$A \rightarrow B$$

Researchers find that the $K_m$ for the substrate A is 4 μM, and the $k_{cat}$ is 20 min$^{-1}$.

(a) In an experiment, [A] = 6 nm, and the initial velocity, $V_0$ was 480 nm min$^{-1}$. What was the [E$_t$] used in the experiment?

(b) In another experiment, [E$_t$] = 0.5 μM, and the measured $V_0$ = 5 μM min$^{-1}$. What was the [A] used in the experiment?

(c) The compound Z is found to be a very strong competitive inhibitor of the enzyme, with an $\alpha$ of 10. In an experiment with the same [E$_t$] as in part (a), but a different [A], an amount of Z is added that reduces the rate $V_0$ to 240 nm min$^{-1}$. What is the [A] in this experiment?

(d) Based on the kinetic parameters given above, has this enzyme evolved to achieve catalytic perfection? Explain your answer briefly, using the kinetic parameter(s) that define catalytic perfection.

**Answer**

(a) Because [S] is much greater than (more than 1000-fold) $K_m$, assume that the measured rate of the reaction reflects $V_{max}$. Use the equation $V_{max} = k_{cat}[E_t]$, and solve for [E$_t$].

$$[E_t] = V_{max}/k_{cat} = 480 \text{ nm min}^{-1}/20 \text{ min}^{-1} = 24 \text{ nm}.$$ 

(b) At this [E$_t$], the calculated $V_{max} = k_{cat}[E_t] = 20 \text{ min}^{-1} \times 0.5 \mu M = 10 \mu M \text{ min}^{-1}$. Recall that $K_m$ equals the substrate concentration at which $V_0 = \frac{1}{2}V_{max}$. The measured $V_0$ is exactly half $V_{max}$, so [A] = $K_m$ = 4 μM.
(c) Given the same $[E_t]$ as in (a), $V_{\text{max}} = 480 \text{ nm} \text{ min}^{-1}$. The $V_0$ is again exactly half $V_{\text{max}}$ ($V_0 = 240 \text{ nm} \text{ min}^{-1}$), so $[A] = \text{the apparent or measured } K_m$. In the presence of an inhibitor with $\alpha = 10$, the measured $K_m = 40 \text{ } \mu\text{M} = [S]$.

(d) No. $k_{\text{cat}}/K_m = 0.33/(4 \times 10^{-6} \text{ m}^{-1} \text{ s}^{-1}) = 8.25 \times 10^4 \text{ m}^{-1} \text{ s}^{-1}$, well below the diffusion-controlled limit.

11. Estimation of $V_{\text{max}}$ and $K_m$ by Inspection

Although graphical methods are available for accurate determination of the $V_{\text{max}}$ and $K_m$ of an enzyme-catalyzed reaction (see Box 6–1), sometimes these quantities can be quickly estimated by inspecting values of $V_0$ at increasing $[S]$. Estimate the $V_{\text{max}}$ and $K_m$ of the enzyme-catalyzed reaction for which the following data were obtained.

<table>
<thead>
<tr>
<th>[S] (m)</th>
<th>$V_0$ (mM/min)</th>
<th>[S] (m)</th>
<th>$V_0$ (mM/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2.5 \times 10^{-6}$</td>
<td>28</td>
<td>$4 \times 10^{-5}$</td>
<td>112</td>
</tr>
<tr>
<td>$4.0 \times 10^{-6}$</td>
<td>40</td>
<td>$1 \times 10^{-4}$</td>
<td>128</td>
</tr>
<tr>
<td>$1 \times 10^{-5}$</td>
<td>70</td>
<td>$2 \times 10^{-3}$</td>
<td>139</td>
</tr>
<tr>
<td>$2 \times 10^{-5}$</td>
<td>95</td>
<td>$1 \times 10^{-2}$</td>
<td>140</td>
</tr>
</tbody>
</table>

Answer Notice how little the velocity changes as the substrate concentration increases by fivefold from 2 to 10 mM. Thus, we can estimate a $V_{\text{max}}$ of 140 mM/min. $K_m$ is defined as the substrate concentration that produces a velocity of $\frac{1}{2}V_{\text{max}}$, or 70 mM. Inspection of the table indicates that this $V_0$ occurs at $[S] = 1 \times 10^{-5}$ M, thus $K_m \approx 1 \times 10^{-5}$ M.

12. Properties of an Enzyme of Prostaglandin Synthesis

Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. They are responsible for producing fever and inflammation and its associated pain. Prostaglandins are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme, a cyclooxygenase, uses oxygen to convert arachidonic acid to PGG$_2$, the immediate precursor of many different prostaglandins (prostaglandin synthesis is described in Chapter 21).

(a) The kinetic data given below are for the reaction catalyzed by prostaglandin endoperoxide synthase. Focusing here on the first two columns, determine the $V_{\text{max}}$ and $K_m$ of the enzyme.

<table>
<thead>
<tr>
<th>[Arachidonic acid] (mM)</th>
<th>Rate of formation of PGG$_2$ (mM/min)</th>
<th>Rate of formation of PGG$_2$ with 10 mg/mL ibuprofen (mM/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>23.5</td>
<td>16.67</td>
</tr>
<tr>
<td>1.0</td>
<td>32.2</td>
<td>25.25</td>
</tr>
<tr>
<td>1.5</td>
<td>36.9</td>
<td>30.49</td>
</tr>
<tr>
<td>2.5</td>
<td>41.8</td>
<td>37.04</td>
</tr>
<tr>
<td>3.5</td>
<td>44.0</td>
<td>38.91</td>
</tr>
</tbody>
</table>

(b) Ibuprofen is an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. Using the data in the first and third columns of the table, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase.
Answer

(a) Calculate the reciprocal values for the data, as in parentheses below, and prepare a double-reciprocal plot to determine the kinetic parameters.

<table>
<thead>
<tr>
<th>[S] (mm) (1/[S] (mm⁻¹))</th>
<th>( \frac{V_0}{(\text{mm/min})} )</th>
<th>( \frac{V_0}{(\text{min/mM})} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (2.0)</td>
<td>23.5 (0.043)</td>
<td>16.67 (0.0600)</td>
</tr>
<tr>
<td>1.0 (1.0)</td>
<td>32.2 (0.031)</td>
<td>25.25 (0.0396)</td>
</tr>
<tr>
<td>1.5 (0.67)</td>
<td>36.9 (0.027)</td>
<td>30.49 (0.0328)</td>
</tr>
<tr>
<td>2.5 (0.40)</td>
<td>41.8 (0.024)</td>
<td>37.04 (0.0270)</td>
</tr>
<tr>
<td>3.5 (0.28)</td>
<td>44.0 (0.023)</td>
<td>38.91 (0.0257)</td>
</tr>
</tbody>
</table>

The intercept on the vertical axis = \(-\frac{1}{V_{\text{max}}}\) and the intercept on the horizontal axis = \(-\frac{1}{K_m}\). From these values, we can calculate \(V_{\text{max}}\) and \(K_m\).

\(-\frac{1}{V_{\text{max}}} = -0.0194\), and \(V_{\text{max}} = 51.5 \text{ mm/min}\)
\(-\frac{1}{K_m} = -1.7\), and \(K_m = 0.59 \text{ mm}\)

(b) Ibuprofen acts as a competitive inhibitor. The double-reciprocal plot (with inhibitor) shows that, in the presence of ibuprofen, the \(V_{\text{max}}\) of the reaction is unchanged (the intercept on the \(1/V_0\) axis is the same) and \(K_m\) is increased (\(-1/K_m\) is closer to the origin).

13. Graphical Analysis of \(V_{\text{max}}\) and \(K_m\) The following experimental data were collected during a study of the catalytic activity of an intestinal peptidase with the substrate glycylglycine:

\[
\text{Glycylglycine} + \text{H}_2\text{O} \rightarrow 2 \text{ glycine}
\]

<table>
<thead>
<tr>
<th>[S] (mm)</th>
<th>Product formed (µmol/min)</th>
<th>[S] (mm)</th>
<th>Product formed (µmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.21</td>
<td>4.0</td>
<td>0.33</td>
</tr>
<tr>
<td>2.0</td>
<td>0.24</td>
<td>8.0</td>
<td>0.40</td>
</tr>
<tr>
<td>3.0</td>
<td>0.28</td>
<td>16.0</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Use graphical analysis (see Box 6–1) to determine the $K_m$ and $V_{\text{max}}$ for this enzyme preparation and substrate.

**Answer** As described in Box 6–1, the standard method is to use $V_0$ versus $[S]$ data to calculate $1/V_0$ and $1/[S]$. Graphing these values gives a Lineweaver-Burk plot. From the best straight line through the data, the intercept on the horizontal axis = $-1/K_m$ and the intercept on the vertical axis = $1/V_{\text{max}}$. From these values, we can calculate $K_m$ and $V_{\text{max}}$:

- $-1/K_m = -0.45$, and $K_m = 2.2 \text{ mm}$
- $-1/V_{\text{max}} = -2.0$, and $V_{\text{max}} = 0.50 \mu\text{mol/min}$

### 14. The Eadie-Hofstee Equation

One transformation of the Michaelis-Menten equation is the Lineweaver-Burk, or double-reciprocal, equation. Multiplying both sides of the Lineweaver-Burk equation by $V_{\text{max}}$ and rearranging gives the Eadie-Hofstee equation:

$$V_0 = (-K_m)\frac{V_0}{[S]} + V_{\text{max}}$$

A plot of $V_0$ vs. $V_0/[S]$ for an enzyme-catalyzed reaction is shown below. The curve labeled “Slope = $-K_m$” was obtained in the absence of inhibitor. Which of the other curves (A, B, or C) shows the enzyme activity when a competitive inhibitor is added to the reaction mixture? Hint: See Equation 6–30.

<table>
<thead>
<tr>
<th>$V_0$ (μmol/min)</th>
<th>$1/V_0$ (min/μmol)</th>
<th>$[S]$ (mm)</th>
<th>$1/[S]$ (mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
<td>4.8</td>
<td>1.5</td>
<td>0.67</td>
</tr>
<tr>
<td>0.24</td>
<td>4.2</td>
<td>2.0</td>
<td>0.50</td>
</tr>
<tr>
<td>0.28</td>
<td>3.6</td>
<td>3.0</td>
<td>0.33</td>
</tr>
<tr>
<td>0.33</td>
<td>3.0</td>
<td>4.0</td>
<td>0.25</td>
</tr>
<tr>
<td>0.40</td>
<td>2.5</td>
<td>8.0</td>
<td>0.13</td>
</tr>
<tr>
<td>0.45</td>
<td>2.2</td>
<td>16.0</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Answer Curve A shows competitive inhibition. \( V_{\text{max}} \) for A is the same as for the normal curve, as seen by the identical intercepts on the \( V_0 \) axis. And, for every value of \([S]\) (until maximal velocity is reached at saturating substrate levels), \( V_0 \) is lower for curve A than for the normal curve, indicating competitive inhibition. Note that as \([S]\) increases, \( V_0/[S] \) decreases, so that \( V_{\text{max}} \)—that is, the \( V_0 \) at the highest (saturating) \([S]\)—is found at the intersection of the curve at the \( y \) axis. Curve C, while also having an identical \( V_{\text{max}} \), shows higher \( V_0 \) values for every \([S]\) (and for every \( V_0/[S] \)) than the normal curve, which is not indicative of inhibition. The lower \( V_{\text{max}} \) for curve B rules out competitive inhibition.

15. The Turnover Number of Carbonic Anhydrase Carbonic anhydrase of erythrocytes (\( M_r 30,000 \)) has one of the highest turnover numbers we know of. It catalyzes the reversible hydration of \( \text{CO}_2 \):

\[
\text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3
\]

This is an important process in the transport of \( \text{CO}_2 \) from the tissues to the lungs. If 10.0 mg of pure carbonic anhydrase catalyzes the hydration of 0.30 g of \( \text{CO}_2 \) in 1 min at 37°C at \( V_{\text{max}} \), what is the turnover number (\( k_{\text{cat}} \)) of carbonic anhydrase (in units of min\(^{-1}\))?

Answer The turnover number of an enzyme is the number of substrate molecules transformed per unit time by a single enzyme molecule (or a single catalytic site) when the enzyme is saturated with substrate:

\[
k_{\text{cat}} = \frac{V_{\text{max}}}{[E_t]}
\]

where \([E_t] = \text{total moles of active sites}\).

We can convert the values given in the problem into a turnover number (min\(^{-1}\)) by converting the weights of enzyme and substrate to molar amounts:

\[
V_{\text{max}} \text{ (moles of } \text{CO}_2/\text{min}) = 0.30 \text{ g/min} \times \frac{44 \text{ g/mol}}{30,000 \text{ g/mol}} = 6.8 \times 10^{-3} \text{ mol/min}
\]

Amount of enzyme (moles) = \( \frac{(10.0 \text{ } \mu g)(1 \text{ g/}10^6 \text{ } \mu g)}{30,000 \text{ g/mol}} = 3.3 \times 10^{-10} \text{ mol} \)

The turnover number is obtained by dividing moles of \( \text{CO}_2/\text{min} \) by moles of enzyme:

\[
k_{\text{cat}} = \frac{6.8 \times 10^{-3} \text{ mol/min}}{3.3 \times 10^{-10} \text{ mol}} = 2.0 \times 10^7 \text{ min}^{-1}
\]

16. Deriving a Rate Equation for Competitive Inhibition The rate equation for an enzyme subject to competitive inhibition is

\[
V_0 = \frac{V_{\text{max}}[S]}{K_m + [S]}
\]

Beginning with a new definition of total enzyme as

\[
[E_t] = [E] + [ES] + [EI]
\]

and the definitions of \( \alpha \) and \( K_I \) provided in the text, derive the rate equation above. Use the derivation of the Michaelis-Menten equation as a guide.

Answer The basic assumptions used to derive the Michaelis-Menten equation still hold. The reaction is at steady state, and the overall rate is determined by

\[
V_0 = k_d[ES]
\]

With the competitive inhibitor, \( I \), now to be added, the goal again is to describe \( V_0 \) in terms of the measurable quantities \([E_t]\), \([S]\), and \([I]\). In the presence of inhibitor,
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\[ [E_i] = [ES] + [E] + [EI] \]  \hspace{1cm} (b)

We first solve for [EI]. As we have seen,

\[ K_i = \frac{[E][I]}{[EI]} \; \text{so} \; [EI] = \frac{[E][I]}{K_i} \]

Substituting for [EI] in (b) gives

\[ [E_i] = [ES] + [E] + \frac{[E][I]}{K_i} \]  \hspace{1cm} (c)

and simplifying gives

\[ [E_i] = [ES] + [E] \left(1 + \frac{[I]}{K_i}\right) = [ES] + [E] \alpha \]  \hspace{1cm} (d)

where \( \alpha \) describes the effect of the competitive inhibitor. [E] in the absence of inhibitor can be obtained from a rearrangement of Equation 6–19 (remembering that \( [E_i] = [ES] + [E] \)), to give

\[ [E] = \frac{[ES]K_m}{[S]} \]  \hspace{1cm} (e)

Substituting (e) into (d) gives

\[ [E_i] = [ES] + \left(\frac{[ES]K_m}{[S]}\right) \alpha \]  \hspace{1cm} (f)

and rearranging and solving for [ES] gives

\[ [ES] = \frac{[E_i][S]}{\alpha K_m + [S]} \]  \hspace{1cm} (g)

Next, substituting (g) into (a), and defining \( k_2[E_i] = V_{max} \), we get the final equation for reaction velocity in the presence of a competitive inhibitor:

\[ V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]} \]

17. Irreversible Inhibition of an Enzyme Many enzymes are inhibited irreversibly by heavy metal ions such as Hg\(^{2+}\), Cu\(^{2+}\), or Ag\(^{+}\), which can react with essential sulfhydryl groups to form mercaptides:

\[ \text{Enz}—\text{SH} + \text{Ag}^+ \longrightarrow \text{Enz}—\text{S—Ag} + \text{H}^+ \]

The affinity of Ag\(^{+}\) for sulfhydryl groups is so great that Ag\(^{+}\) can be used to titrate —SH groups quantitatively. To 10.0 mL of a solution containing 1.0 mg/mL of a pure enzyme, an investigator added just enough AgNO\(_3\) to completely inactivate the enzyme. A total of 0.342 mmol of AgNO\(_3\) was required. Calculate the minimum molecular weight of the enzyme. Why does the value obtained in this way give only the minimum molecular weight?

**Answer** An equivalency exists between millimoles of AgNO\(_3\) required for inactivation and millimoles of —SH group and thus, assuming one —SH group per enzyme molecule, millimoles of enzyme:

\[ 0.342 \times 10^{-3} \text{ mmol} = \frac{(1.0 \text{ mg/mL})(10.0 \text{ mL})}{(\text{minimum } M_r)(\text{mg/mmol})} \]

Thus, the minimum \( M_r = \frac{(1.0 \text{ mg/mL})(10.0 \text{ mL})}{0.342 \times 10^{-3} \text{ mmol}} = 2.9 \times 10^4 = 29,000 \)

This is the minimum molecular weight because it assumes only one titratable —SH group per enzyme molecule.
18. Clinical Application of Differential Enzyme Inhibition Human blood serum contains a class of enzymes known as acid phosphatases, which hydrolyze biological phosphate esters under slightly acidic conditions (pH 5.0):

\[ \text{R-} \text{O-PO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{R-} \text{OH} + \text{HO-PO}_3^{2-} \]

Acid phosphatases are produced by erythrocytes, the liver, kidney, spleen, and prostate gland. The enzyme of the prostate gland is clinically important because its increased activity in the blood can be an indication of prostate cancer. The phosphatase from the prostate gland is strongly inhibited by tartrate ion, but acid phosphatases from other tissues are not. How can this information be used to develop a specific procedure for measuring the activity of the acid phosphatase of the prostate gland in human blood serum?

**Answer** First, measure the total acid phosphatase activity in a blood sample in units of \( \mu \text{mol} \) of phosphate ester hydrolyzed per mL of serum. Next, remeasure this activity in the presence of tartrate ion at a concentration sufficient to completely inhibit the enzyme from the prostate gland. The difference between the two activities represents the activity of acid phosphatase from the prostate gland.

19. Inhibition of Carbonic Anhydrase by Acetazolamide Carbonic anhydrase is strongly inhibited by the drug acetazolamide, which is used as a diuretic (i.e., to increase the production of urine) and to lower excessively high pressure in the eye (due to accumulation of intraocular fluid) in glaucoma. Carbonic anhydrase plays an important role in these and other secretory processes because it participates in regulating the pH and bicarbonate content of several body fluids. The experimental curve of initial reaction velocity (as percentage of \( V_{\text{max}} \)) versus \([S]\) for the carbonic anhydrase reaction is illustrated below (upper curve). When the experiment is repeated in the presence of acetazolamide, the lower curve is obtained. From an inspection of the curves and your knowledge of the kinetic properties of competitive and mixed enzyme inhibitors, determine the nature of the inhibition by acetazolamide. Explain your reasoning.

**Answer** The graph gives us several pieces of information. First, the inhibitor prevents the enzyme from achieving the same \( V_{\text{max}} \) as in the absence of inhibitor. Second, the overall shape of the two curves is very similar: at any \([S]\) the ratio of the two velocities (\( \frac{V}{V_{\text{max}}} \)) is the same. Third, the velocity does not change very much above \([S] = 1 \text{ mm}\), so at much higher \([S]\) the observed velocity is essentially \( V_{\text{max}} \) for each curve. Fourth, if we estimate the \([S]\) at which \( \frac{1}{2} V_{\text{max}} \) is achieved, this value is nearly identical for both curves. Noncompetitive inhibition, a special form of mixed inhibition that is rarely observed, alters the \( V_{\text{max}} \) of enzymes but leaves \( K_m \) unchanged. Thus, acetazolamide acts as a noncompetitive (mixed) inhibitor of carbonic anhydrase.
20. The Effects of Reversible Inhibitors Derive the expression for the effect of a reversible inhibitor on observed \( K_m \) (apparent \( K_m / \alpha' \)). Start with Equation 6–30 and the statement that apparent \( K_m \) is equivalent to the \([S]\) at which \( V_0 = V_{max}/2\alpha' \).

**Answer** Equation 6–30 is

\[
V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}
\]

Or \( V_0 = V_{max} \times [S]/(\alpha K_m + \alpha'[S]) \). Thus, the \([S]\) at which \( V_0 = V_{max}/2\alpha' \) is obtained when all the terms on the right side of the equation except \( V_{max} \) equal \( \frac{1}{2} \alpha' \):

\[
[S]/(\alpha K_m + \alpha'[S]) = \frac{1}{2} \alpha'
\]

We can now solve this equation for \([S]\):

\[
2\alpha'[S] = \alpha K_m + \alpha'[S]
\]

\[
2\alpha'[S] - \alpha'[S] = \alpha K_m
\]

\[
\alpha'[S] = \alpha K_m
\]

\[
[S] = \alpha K_m / \alpha'
\]

Thus, observed \( K_m = \alpha K_m / \alpha' \).

21. pH Optimum of Lysozyme The active site of lysozyme contains two amino acid residues essential for catalysis: Glu\(^{35}\) and Asp\(^{52}\). The \( pK_a \) values of the carboxyl side chains of these residues are 5.9 and 4.5, respectively. What is the ionization state (protonated or deprotonated) of each residue at pH 5.2, the pH optimum of lysozyme? How can the ionization states of these residues explain the pH-activity profile of lysozyme shown below?

**Answer** At a pH midway between the two \( pK_a \) values (pH 5.2), the side-chain carboxyl group of Asp\(^{52}\), with the lower \( pK_a \) (4.5), is mainly deprotonated (—COO\(^{-}\)), whereas Glu\(^{35}\), with the higher \( pK_a \) (5.9; the stronger base), is protonated (—COOH). At pH values below 5.2, Asp\(^{52}\) becomes protonated and the activity decreases. Similarly, at pH values above 5.2, Glu\(^{35}\) becomes deprotonated and the activity also decreases. The pH-activity profile suggests that maximum catalytic activity occurs at a pH midway between the \( pK_a \) values of the two acidic groups, when Glu\(^{35}\) is protonated and Asp\(^{52}\) is deprotonated.

22. Working with Kinetics Go to the Living Graphs for Chapter 6.

(a) Using the Living Graph for Equation 6–9, create a \( V \) versus \([S]\) plot. Use \( V_{max} = 100 \ \mu\text{M} \ \text{s}^{-1} \), and \( K_m = 10 \ \mu\text{M} \). How much does \( V_0 \) increase when \([S]\) is doubled, from 0.2 to 0.4 \( \mu\text{M} \)? What is \( V_0 \) when \([S]\) = 10 \( \mu\text{M} \)? How much does the \( V_0 \) increase when \([S]\) increases from 100 to 200 \( \mu\text{M} \)? Observe how the graph changes when the values for \( V_{max} \) or \( K_m \) are halved or doubled.
(b) Using the Living Graph for Equation 6–30 and the kinetic parameters in (a), create a plot in which both $\alpha$ and $\alpha'$ are 1.0. Now observe how the plot changes when $\alpha = 2.0$; when $\alpha' = 3.0$; and when $\alpha = 2.0$ and $\alpha' = 3.0$.

(c) Using the Living Graphs for Equation 6–30 and the Lineweaver-Burk equation in Box 6–1, create Lineweaver-Burk (double-reciprocal) plots for all the cases in (a) and (b). When $\alpha = 2.0$, does the $x$ intercept move to the right or to the left? If $\alpha = 2.0$ and $\alpha' = 3.0$, does the $x$ intercept move to the right or to the left?

**Answer**

(a) When $[S]$ increases from 0.2 to 0.4 $\mu$M, $V_0$ increases by a factor of 1.96. When $[S] = 10$ $\mu$M, $V_0 = 50$ $\mu$M s$^{-1}$. When $[S]$ increases from 100 to 200 $\mu$M, $V_0$ increases by a factor of 1.048.

(b) When $\alpha = 2.0$, the curve is shifted to the right as the $K_m$ is increased by a factor of 2. When $\alpha' = 3.0$, the asymptote of the curve (the $V_{max}$) declines by a factor of 3. When $\alpha = 2.0$ and $\alpha' = 3.0$, the curve briefly rises above the curve where both $\alpha$ and $\alpha' = 1.0$, due to a decline in $K_m$. However, the asymptote is lower because $V_{max}$ declines by a factor of 3.

(c) When $\alpha = 2.0$, the $x$ intercept moves to the right. When $\alpha = 2.0$ and $\alpha' = 3.0$, the $x$ intercept moves to the left.

**Data Analysis Problem**

23. Exploring and Engineering Lactate Dehydrogenase Examining the structure of an enzyme results in hypotheses about the relationship between different amino acids in the protein's structure and the protein's function. One way to test these hypotheses is to use recombinant DNA technology to generate mutant versions of the enzyme and then examine the structure and function of these altered forms. The technology used to do this is described in Chapter 9.

One example of this kind of analysis is the work of Clarke and colleagues on the enzyme lactate dehydrogenase, published in 1989. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate with NADH to form lactate (see Section 14.3). A schematic of the enzyme's active site is shown below; the pyruvate is in the center.
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The reaction mechanism is similar to many NADH reductions (Fig. 13–24); it is approximately the reverse of steps 2 and 3 of Figure 14–7. The transition state involves a strongly polarized carbonyl group of the pyruvate molecule as shown below:

(a) A mutant form of LDH in which Arg$^{109}$ is replaced with Gln shows only 5% of the pyruvate binding and 0.07% of the activity of wild-type enzyme. Provide a plausible explanation for the effects of this mutation.

(b) A mutant form of LDH in which Arg$^{171}$ is replaced with Lys shows only 0.05% of the wild-type level of substrate binding. Why is this dramatic effect surprising?

(c) In the crystal structure of LDH, the guanidinium group of Arg$^{171}$ and the carboxyl group of pyruvate are aligned as shown in a co-planar “forked” configuration. Based on this, provide a plausible explanation for the dramatic effect of substituting Arg$^{171}$ with Lys.

(d) A mutant form of LDH in which Ile$^{250}$ is replaced with Gln shows reduced binding of NADH. Provide a plausible explanation for this result.

Clarke and colleagues also set out to engineer a mutant version of LDH that would bind and reduce oxaloacetate rather than pyruvate. They made a single substitution, replacing Gln$^{102}$ with Arg; the resulting enzyme would reduce oxaloacetate to malate and would no longer reduce pyruvate to lactate. They had therefore converted LDH to malate dehydrogenase.

(e) Sketch the active site of this mutant LDH with oxaloacetate bound.

(f) Provide a plausible explanation for why this mutant enzyme now “prefers” oxaloacetate instead of pyruvate.

(g) The authors were surprised that substituting a larger amino acid in the active site allowed a larger substrate to bind. Provide a plausible explanation for this result.

Answer

(a) In the wild-type enzyme, the substrate is held in place by a hydrogen bond and an ion-dipole interaction between the charged side chain of Arg$^{109}$ and the polar carbonyl of pyruvate. During catalysis, the charged Arg$^{109}$ side chain also stabilizes the polarized carbonyl transition state. In the mutant, the binding is reduced to just a hydrogen bond, substrate binding is weaker, and ionic stabilization of the transition state is lost, reducing catalytic activity.

(b) Because Lys and Arg are roughly the same size and have a similar positive charge, they probably have very similar properties. Furthermore, because pyruvate binds to Arg$^{171}$ by (presumably) an ionic interaction, an Arg to Lys mutation would probably have little effect on substrate binding.

(c) The “forked” arrangement aligns two positively charged groups of Arg residues with the negatively charged oxygens of pyruvate and facilitates two combined hydrogen-bond and ion-dipole interactions. When Lys is present, only one such combined hydrogen-bond and ion-dipole interaction is possible, thus reducing the strength of the interaction. The positioning of the substrate is less precise.

(d) Ile$^{250}$ interacts hydrophobically with the ring of NADH. This type of interaction is not possible with the hydrophilic side chain of Gln.
(e) The structure is shown below.

(f) The mutant enzyme rejects pyruvate because pyruvate's hydrophobic methyl group will not interact with the highly hydrophilic guanidinium group of Arg\(^{102}\). The mutant binds oxaloacetate because of the strong ionic interaction between the Arg\(^{102}\) side chain and the carboxyl of oxaloacetate.

(g) The protein must be flexible enough to accommodate the added bulk of the side chain and the larger substrate.

References