Appendix

1. Derivation of the Rate Equation by King–Altman’s Method

The derivation of the rate equation for the Michaelis–Menten mechanism is not hard. However, to derive the rate equation for the complicated enzyme-catalyzed reaction, including various enzyme species, is not easy. King and Altman introduced a simple schematic method applicable for various enzyme-catalyzed reactions [1–3]. For simplicity, apply the method to a following simple mechanism:

\[
E + S \xrightleftharpoons[k_1]{k_{-1}} ES \xrightleftharpoons[k_{2}]{k_{-2}} EP \xrightleftharpoons[k_3]{k_{-3}} E + P \tag{A.1}
\]

Draw the polygon having one enzyme species on each top (basic pattern). Therefore, at least three enzyme species are required for this mechanism.

Basic Pattern

\[
\begin{align*}
&S \xrightarrow[k_1]{k_{-1}} E \xrightarrow[k_{2}]{k_{-2}} EP \xrightarrow[k_3]{k_{-3}} E + P \\
&ES \xrightarrow[k_1]{k_{-1}} E \xrightarrow[k_{2}]{k_{-2}} EP \xrightarrow[k_3]{k_{-3}} E + P \\
&E \xrightarrow[k_{-3}]{k_3} EP \xrightarrow[k_{-2}]{k_2} ES \xrightarrow[k_{-1}]{k_1} E
\end{align*}
\]

(1) Write an arrow on each side and the rate constant for the respective reaction near the arrow. When a ligand is involved in the reaction, write the rate constant multiplied with the concentration of the ligand.

(2) Write all possible polygons without one side as above (calculation pattern).
On the basis of the patterns, determine \([E]/e_0\), \([\text{ES}]/e_0\), and \([\text{EP}]/e_0\). Here, \(e_0\) is the total concentration of the enzyme species involved. For example, \([E]\) can be determined by multiplying the rate constants going toward the enzyme species “E” for each calculation pattern:

\[
\frac{[E]}{e_0} = \frac{(k_{-1}k_{+2} + k_{+1}k_{-2} + k_{+2}k_{-3})}{\Sigma}
\]  
(A.2)

Similarly,

\[
\frac{[\text{ES}]/e_0}{} = \frac{(k_{+1}k_{-2}[S] + k_{+2}k_{-3}[P] + k_{+3}k_{-3}[S])}{\Sigma}
\]  
(A.3)

\[
\frac{[\text{EP}]/e_0}{} = \frac{(k_{+1}k_{-2}[S] + k_{+2}k_{-3}[P] + k_{+3}k_{-3}[P])}{\Sigma}
\]  
(A.4)

where \(\Sigma\) is equal to the sum of the numerators of Eqs. A.2 to A.4.

\[
\Sigma = (k_{-1}k_{+3} + k_{+1}k_{-2} + k_{+2}k_{+3}) + (k_{+1}k_{-2}[S] + k_{+2}k_{-3}[P] + k_{+3}k_{-3}[S])
\]

\[
+ (k_{+1}k_{-2}[S] + k_{+2}k_{-3}[P] + k_{+3}k_{-3}[P])
\]

Then the rate of reaction, \(v\), is

\[
v/e_0 = \frac{[k_{+3}[\text{EP}] - k_{-3}[E][P]]}{\Sigma}
\]

When we measure the initial rate, \([P]\) is zero, then

\[
v/e_0 = \frac{k_{+3}[\text{EP}]}{\Sigma} = \frac{k_{+1}k_{+3}[S]}{\{(k_{+1}k_{+3} + k_{-1}k_{+2} + k_{+2}k_{+3})
\]

\[
+ (k_{+1}k_{+2}[S] + k_{+1}k_{+3}[S] + k_{+1}k_{+2}[S])\}
\]

\[
v = \frac{k_{+1}k_{+2}k_{+3}[S]e_0}{\{(k_{-1}k_{+3} + k_{+1}k_{-2} + k_{+2}k_{+3}) + k_{+1}(k_{+2} + k_{+3})[S]\}}
\]

References

2. Physical Constants

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<tr>
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<th>Symbol</th>
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<tbody>
<tr>
<td>Planck’s constant</td>
<td>$h$</td>
<td>$6.6261 \times 10^{-34}$ J s</td>
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<tr>
<td>Avogadro constant</td>
<td>$N_A$</td>
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<tr>
<td>Boltzmann constant</td>
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<td>Gas constant</td>
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<td>Faraday constant</td>
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<td>$9.6485 \times 10^4$ C mol$^{-1}$</td>
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*The International System of Units.

3. Conversion of Units

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<td>$1 \text{ N m}$</td>
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<tr>
<td></td>
<td>$1$ J</td>
<td>$1$ kg m$^2$ s$^{-2}$</td>
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4. Prefix of Numbers and Alphabets in Greek

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</tr>
<tr>
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Alphabet: Upper-case is not shown.
5. Useful Software and Data Banks

**Jmol**: an open-source Java viewer for chemical structures in 3D; http://jmol.sourceforge.net/

**PyMOL**: a software tool for the analysis and visualization of protein in 3D; http://www.pymol.org/

**Caver**: software tool for the analysis and visualization of tunnels and channels in protein structures; http://www.caver.cz/

**Chimera**: software for the visualization and analysis of molecular structures; good for matching two protein structures; http://www.cgl.uchsf.edu/chimera/

**Protein data bank**: http://www.pdbj.org/; http://www.ebi.ac.uk/msd/

**DNA data bank Japan (DDBJ)**: http://www.ddbj.nig.ac.jp/index-j.html

6. Genetic Code

<table>
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<tr>
<th>First position (5'-end)</th>
<th>Second position</th>
<th>Third position (3'-end)</th>
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<td>Tyr</td>
</tr>
<tr>
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<td>Tyr</td>
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<td>Ser</td>
<td>Stop</td>
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<td>Leu</td>
<td>Ser</td>
<td>Stop^b</td>
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<tr>
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<td>Leu</td>
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<td>Glu</td>
</tr>
<tr>
<td>Val</td>
<td>Ala</td>
<td>Glu</td>
</tr>
</tbody>
</table>

In some proteins, stop codons code amino acids: ^aU/TGA codes selenocystein, and ^bU/TAG pyrolysine. See Chapter 6.
Solutions

Answers have been given for important and hard problems. Readers are strongly recommended to solve problems independently and then read the answer.

Chapter 2

1. Figure 2.2 is a plot \( \frac{v}{V} \) vs. \( \log s \). Therefore, Eq. 2.16 must be changed to a function of \( \log s \):

\[
\frac{v}{V} = \frac{10^x}{10^x + K_s}
\]

where \( x = \log s \). Solve the following differential equation at \( \frac{v}{V} = 0.5 \):

\[
\frac{d(\frac{v}{V})}{dx} = \frac{d}{dx} \left( \frac{10^x}{10^x + K_s} \right)
\]

The final equation is

\[
\frac{d(\frac{v}{V})}{d(\log s)} = \frac{2.303 \cdot K_s s}{(s + K_s)^2}
\]

At \( \frac{v}{V} = 0.5 \), \( s = K_s \), we can obtain the slope, \( 2.303/4 \).

3. The lag period is the time \( t \) when \( p = 0 \) in Eq. 2.26. Thus, \( t = 1/k \).

4. Apply Eq. (2.23) 98.2%.

Chapter 3

2. Apply the steady state for [ES\(_1\)-EP\(_1\)], [F], and [FS\(_2\)-FP\(_2\)], respectively, and derive the rate \( (v) \) of the enzymatic reaction using
Chapter 4

1. Differentiation of Eq. 4.7 leads to

\[
\frac{dV}{dH} = \frac{-\tilde{V}}{(1 + \frac{H}{K_1} + \frac{K_2}{H})^2} \left( \frac{H^2 - K_1 K_2}{H^2 K_1} \right)
\]

At the optimum pH (the proton concentration, \(H_{op}\)),

\[
dV/dH = 0
\]

Thus, \(H_{op}^2 = K_1 K_2\) (Eq. 4.12).

The rate \(V_{op}\) at \(H_{op}\) is determined to be given by replacing \(H\) into \(H_{op}\) in Eq. 4.7:

\[
V_{op} = \frac{\tilde{V}}{\left(1 + \sqrt{K_1 K_2} / K_1 + \sqrt{K_2 / K_1}\right)} = \frac{\tilde{V}}{1 + 2 \left(\frac{K_2}{\sqrt{K_1}}\right)}
\]

Then

\[
\frac{V}{V_{op}} = \frac{1 + 2 \sqrt{\frac{K_2}{K_1}}}{\left(1 + \frac{H}{K_1} + \frac{K_2}{H}\right)}
\]

From this equation, \(H_1\) and \(H_2\) are calculated by introducing \(V/V_{op} = 1/2\); then

\[
H^2 - (4\sqrt{K_1 K_2} + K_1)H + K_1 K_2 = 0
\]

Assuming that a solution to the equation is \(H_1\) and \(H_2\),

\[(H - H_1)(H - H_2) = 0\]

Then,

\[H_1 + H_2 = 4\sqrt{K_1 K_2} + K_1 = K_1 + 4H_{op}\]
From Eq. 4.27, assuming $T = 298$ K, the free energy of activation can be determined by introducing 193 s$^{-1}$ into $k_f$, then the activation entropy is calculated.

3. From Eq. 4.37 and the slope in Fig. 4.10,

$$\Delta V^\ddagger = -RT \frac{d(2.303 \log k)}{dp}$$

$$= -2.303 RT \frac{d(\log k)}{dp}$$

$$= -2.303 \cdot 0.0831 \cdot 10^3 (\text{ml bar} \text{K mol}^{-1}) \cdot 298(K) \cdot 0.415 \times 10^{-3} (\text{bar}^{-1})$$

$$= -23.7 \text{ml}$$

Chapter 5

$$\lambda_t - \lambda_\infty = (\lambda_0 - \lambda_\infty) \exp (-kt) \quad (\text{SM5.1})$$

$$\lambda_t' - \lambda_\infty = (\lambda_0 - \lambda_\infty) \exp \{-k(t + \Delta)\} \quad (\text{SM5.2})$$

Eq. SM5.1 – Eq. SM5.2,

$$\lambda_t - \lambda_t' = (\lambda_0 - \lambda_\infty) \exp (-kt) \{1 - \exp(-k\Delta)\}$$

$$\ln (\lambda_t - \lambda_t') = -kt + \ln [(\lambda_0 - \lambda_\infty)\{1 - \exp(-k\Delta)\}]$$

Chapter 6

1. Let’s start with simple examples. One disulfide bond is possible for two SH groups. For four groups, $C(4,2)/2!$ (i.e., 3) combinations of disulfide bond location is possible, where $C(a,b) = a!/(a–b)!b!$. For six groups, $C(6,2) \times C(4,2)/3!$ (i.e., 15) combinations. Thus, for eight SH groups, $C(8,2) \times C(6,2) \times C(4,2)/4!$ (i.e., 105) combinations. In the RNAase A, the native enzyme has only one in 105 combinations. Consider how cells select one combination?

2. The indole ring of Trp residue is stimulated by absorbing a light energy to the activated state. Then the activated indole releases an energy to return the initial low energy state. Usually the energy is released as fluorescence. By binding with
pyruvate, Trp residue on the loop moves to bind with amino acid residues; thus, the energy on the indole is transferred to the residues. Therefore, the fluorescence is decreased.

Chapter 7

2. The interconversion between isomers of organic compounds is called tautomerization. Each isomer is called tautomer. The keto-enol tautomerization may be representative:

\[
\begin{align*}
\text{enol} & \quad \leftrightarrow \quad \text{keto} \\
\end{align*}
\]

A proton migrates in the reaction. In the amino-imino tautomerization of TPP, N1' and N4' are involved in tautomerization: A relay of a proton between N1' and N4' through the pyrimidine ring interconverts between the amino and imino tautomers. In the TPP-dependent enzymes, the imino tautomer, 1',4'-iminopyrimidine, which deprotonates the C2-H, thus, forming C2 carbanion as described in the text. See ref. 7 in Chapter 7 for further information.

Chapter 8

2. Magnesium ions are treated as substrate and product. Mg₁, Mg₂, and Mg₁₂ represent that Mg ions are binding at the Mg₁, Mg₂ sites, and both sites, respectively. S and P represent the substrate peptide and the phosphorylated product, respectively. C represents the catalytic subunit of PKA.
3.4. From the assumptions,

\[ \frac{es}{x} = \frac{(e_0 - x - y)s}{x} = \frac{k_{-1}}{k_{s1}} = K \]  

(SM8.1)

Then,

\[ x = \frac{s}{K + s} (e_0 - y) \]  

(SM8.2)

From the scheme given,

\[ \frac{dy}{dt} = k_{s2}x - k_{s3}y \]  

(SM8.3)

Substituting Eq. SM8.2 into Eq. SM8.3,

\[ \frac{dy}{dt} = k_{s2} \left\{ \frac{s}{K + s} (e_0 - y) \right\} - k_{s3}y = \frac{k_{s2}se_0}{K + s} - \left( \frac{k_{s2}s}{K + s} + k_{s3} \right) y \]  

(SM8.4)

Integration of Eq. SM8.4 from time 0 to \( t \) gives

\[ y = \frac{k_{s2}se_0}{\lambda(K + s)} (1 - e^{-\lambda t}) \]  

(SM8.5)

where,

\[ \lambda = k_{s3} + \frac{k_{s2}s}{K + s} \]

By applying Eq. SM8.2, the rate of formation of \( P_1 \) is

\[ \frac{dp_1}{dt} = k_{s2}x = k_{s2} \frac{s}{K + s} (e_0 - y) \]  

(SM8.6)

Substituting Eq. SM8.5 into Eq. SM8.6, and the resulting equation is integrated from time 0 to \( t \),
\[ p_1 = \frac{k_{s_2} k_{s_3}}{\lambda} s e_0 t + \frac{k_{s_2}^2}{\lambda^2} \left( \frac{s}{K + s} \right)^2 (1 - e^{-\lambda t}) \]

\[ = \frac{k_{s_2} k_{s_3} e_0 s / (k_{s_2} + k_{s_3})}{s + \frac{k_{s_3} K}{(k_{s_2} + k_{s_3})}} t + \left( \frac{k_{s_2}}{k_{s_2} + k_{s_3}} \right)^2 \left( \frac{s}{k_{s_3} K / (k_{s_2} + k_{s_3}) + s} \right)^2 e_0 (1 - e^{-\lambda t}) \]  

(SM8.7)

\[ p_1 = v_0 t + \pi (1 - e^{-\lambda t}) \]  

(SM8.8)

where

\[ v_0 = \frac{k_{s_2} k_{s_3} e_0 s / (k_{s_2} + k_{s_3})}{s + K_m}; \quad \pi = \left( \frac{k_{s_2}}{k_{s_2} + k_{s_3}} \right)^2 \left( \frac{s}{K_m / s + s} \right)^2 e_0 \]  

(SM8.9)

Here, \( K_m = \frac{k_{s_3} K}{(k_{s_2} + k_{s_3})} \)

In the second equation of Eq. SM8.9, the reciprocal of square root of both side,

\[ \frac{1}{\sqrt{\pi}} = \left( 1 + \frac{k_{s_3}}{k_{s_2}} \left( 1 + \frac{K_m}{s} \right) \right) \frac{1}{\sqrt{e_0}} \]

under the condition, \( k_{s_2} \gg k_{s_3} \)

\[ \frac{1}{\sqrt{\pi}} = \frac{1}{\sqrt{e_0}} \left( 1 + \frac{K_m}{s} \right) \]

Thus, the crossing point of the ordinate gives the active concentration of enzyme as shown in Box 8.2. The above derivations are described in papers and books such as [1–4]. Probably, the derivation in [2] may be easy to understand.

References


**Chapter 9**

1. The DEAE group is positively charged at pH 7.0. Therefore, the negatively charged group is able to bind with the DEAE group. The binding strength is dependent on the total charge of proteins.

Proteins having lower pl than the pH of the buffer; thus, bovine serum albumin and human hemoglobin have negative charges at pH 7.0 and bind with the cellulose. The binding is stronger with bovine serum albumin than human hemoglobin. On the other hand, cytochrome c is positively charged, then does not bind with the cellulose. Order is cytochrome c, human hemoglobin, and bovine serum albumin.

2. NaCl dissociates into Na⁺ and Cl⁻. These ions neutralize the charged portions of proteins; thus, interactions with the DEAE cellulose decrease to dissociate proteins from the cellulose.

**Chapter 10**

2. The steady state concentrations of the enzyme species, Eox-S, Ered-Im, Ered-Im-O₂ are constant, and the total concentration of enzyme is

\[ e_0 = [E_{ox}] + [E_{ox-S}] + [E_{red-Im}] + [E_{red-Im-O_2}] \]

and the rate of the catalytic reaction is

\[ v = d[P]/dt = k_4[[E_{red-Im-O_2}] \]

From these, we will derive Eq. 10.6.

3. \[ k_H = A_H \exp\left(-E_{aH}/RT\right) \]  
   \[ k_D = A_D \exp\left(-E_{aD}/RT\right) \]  
   \[ k_H/k_D = (A_H/A_D) \exp\left\{-E_{aH} + E_{aD}\right)/RT\} \]
As $E_{aD} - E_{aH} = 4.8 \text{ kJ/mol}$ and $T = 298 \text{ K}$, $k_H/k_D$ is calculated to be 6.94, since $A_{H}/A_{D} = 1$.

4. In the region IV of Fig. 10.4, the activationless ground state H-tunneling occurs. Using Eq. 1.3,

\[
\ln k_H = -\frac{E_{aH}}{RT} + \ln A_H \quad \text{(SM10.4)}
\]

\[
\ln k_D = -\frac{E_{aD}}{RT} + \ln A_D \quad \text{(SM10.5)}
\]

Subtracting Eq. SM10.5 from Eq. SM10.4 gives

\[
\ln k_H - \ln k_D = \left(-\frac{E_{aH} + E_{aD}}{RT}\right) + \ln A_H - \ln A_D
\]

As $(-E_{aH} + E_{aD})$ is zero under the conditions,

$k_H/k_D = A_H/A_D$. 