1. (4 pts.) Draw the structure of the last two nucleotides of a terminated single-stranded DNA polymer that could be found in a DNA sequencing reaction that contained ddCTP. Draw the base of the next-to-last nucleotide as adenine (A). Include the numbering systems of the sugars and bases, and begin drawing the structure at the oxygen atom attached to the 5' carbon atom of the sugar of the next-to-last nucleotide.

![Structure of nucleotides](image)

2. Taking a much needed break from your medical studies, you moonlight in the Sarge laboratory studying a newly discovered human disease called Watson-Crick Syndrome. The hallmark symptom of this horrible disease is the complete inability to draw nucleic acid structures. Sadly, people afflicted with Watson-Crick Syndrome can never aspire to become Biochemistry Professors or get through the first year of medical school. Sad indeed.

A. (2 pts.) You just finished sequencing the gene for this disease and found that a single nucleotide mutation is responsible for the defect. However, you are disappointed to find that it won’t be possible to use Restriction Fragment Length Polymorphism (RFLP) analysis to test patients for this disease. What did you find out?

You found out that the nucleotide mutation in the gene for this disease did not result in a change in restriction enzyme digestion site for any known restriction enzymes, and thus no restriction enzyme available will digest this sequence differently from the normal DNA sequence. Since this is the basis for RFLP analysis, you can’t use this technique for identifying people with this mutation.
B. (3 pts.) Your spirits pick up when the ever-helpful Dr. Sarge reminds you of another technique you might use to rapidly screen patients for this disease (that we talked about in class). Describe the steps employed in this technique.

1. Synthesize two different DNA oligonucleotide probes, one whose sequence exactly matches that of the mutant gene in the region of the mutation, the other having a sequence that matches the corresponding region of the normal (unmutated, wildtype) gene. Label the probes with either radioactivity (e.g. 32P) or fluorescent tag so you can detect them.

2. Isolate DNA from people suspected of having this mutation as well as people who are not suspected of having this mutation (for comparison), and place duplicate spots of these DNA samples on two different pieces of special filter paper.

3. Incubate one of the DNA spot sets with the probe that is specific for the mutant gene sequence and the other set of DNA spots with the probe specific for the normal gene sequence. After washing away unbound probes, expose to X-ray film or fluorescence detection method.

3. (3 pts.)

Part 1: Mrs. Smith came to see you. She wants to know if she has to lose weight. She weighs in at 150 pounds and is 5 feet 2 inches tall. She tells you her blood pressure is also high. She is 35 years of age. Her body mass index (BMI) is: Circle the correct answer (1 pt.)

- a) 29.3
- b) 31.2
- c) 27.5
- d) 40.7

Part 2: Does she need to lose weight? Yes No (Circle the correct response) (1 pt.)

Part 3: How many kilocalories does she need per day to maintain her current weight using the following Harris-Benedict equation for basal energy expenditure (BEE)? She is 35 years old and has an activity factor of 1.3:

\[ \text{BEE} = 655.1 + [9.66 \times W] + [1.85 \times H] - [4.68 \times A] \]

Circle the correct answer (1 pt.)

- a) 1441.3
- b) 1768.9
- c) 2299.5
- d) 1873.6
4. (1 pt.) Which one of the compounds below could be used to prepare two different solutions, one buffered at pH 6.0 and the other at pH 9.0?

C. Imidazole @ pH 6 and NH_{2} @ pH 9

a. \( \text{NH}_{2}-C-\text{NH}-(\text{CH}_{2})_{3}-C-\text{COOH} \)
d. \( \text{NH}_{2}-\text{CH}_{2}-\text{COOH} \)

b. \( \text{NH}_{2}-C-\text{CH}_{2}-C-\text{COOH} \)
e. \( \text{NH}_{2}-(\text{CH}_{2})_{4}-C-\text{COOH} \)

c. \[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{C} \\
\text{O} \\
\text{N} \\
\text{H} \\
\end{array}
\]  

5. (2 pts.) For the ionization of phosphoric acid shown below:

\[
\begin{align*}
\text{H}_{3}\text{PO}_{4} & \rightleftharpoons \text{H}^{+} + \text{H}_{2}\text{PO}_{4}^{-} \\
& \rightleftharpoons \text{H}^{+} + \text{HPO}_{4}^{2-} \\
& \rightleftharpoons \text{H}^{+} + \text{PO}_{4}^{3-}
\end{align*}
\]

\[ \text{Ka}_{1} = 7.9 \times 10^{-3} \quad \text{Ka}_{2} = 1.58 \times 10^{-7} \quad \text{Ka}_{3} = 2.0 \times 10^{-13} \]

You are preparing a solution buffered at pH = 2.4 containing [0.3 M] of all phosphate species, where appropriate as the sodium salt. Please label the diagram above to indicate the conjugate acid and conjugate base forms that would form a buffer at this pH and calculate the weight of conjugate acid and conjugate base forms that would be dissolved to give 1 liter of this solution based on the formula weights given below:

\( \text{H}_{3}\text{PO}_{4} \) (98 gms/mole), \( \text{NaH}_{2}\text{PO}_{4} \) (120 gms/mole), \( \text{Na}_{2}\text{HPO}_{4} \) (142 gms/mole), \( \text{Na}_{3}\text{PO}_{4} \) (164 gms/mole).

At pH = 2.4, the 1\text{st} ionization of \( \text{H}_{3}\text{PO}_{4} \) is involved. pKa is calculated from the value given for Ka\text{1} since pKa = - Log Ka = - Log 7.9 \times 10^{-3} = 0.9 – 3 = 2.1. The conjugate acid, HA is phosphoric acid and the conjugate base is sodium dihydrogen phosphate (\( \text{NaH}_{2}\text{PO}_{4} \)).

Henderson-Hasselbalch equation gives

\[
pH = pKa + \log \left( \frac{[A^{-}]}{[HA]} \right) \quad \text{or} \quad 2.4 = 2.1 + \log \left( \frac{[A^{-}]}{[HA]} \right)
\]

and \( \frac{[A^{-}]}{[HA]} = 2 \) and \( [A^{-}] = 2[HA] \)

The total concentration of Pi is 0.3 M. Therefore, \([HA] + [A^{-}] = 0.3 \text{ M}\)

By substitution, \([HA] + 2[HA] = 0.3\text{M} \) or \([HA] = 0.1 \text{ M} \). \([A^{-}] = 0.2 \)

For 1 liter of solution, 0.1 Moles/L \( \text{H}_{3}\text{PO}_{4} \) = 0.1 moles x 98 gms/mol = 9.8 gms.

Similarly, 0.2 M \( \text{NaH}_{2}\text{PO}_{4} \) x 1 L = 0.2 moles x 120 gms/mol = 24 gms.
6. (2 pts.) A compound has a pKa = 6.8. Starting with 100 ml of a 1 M solution of this compound prepared, at pH 7.4, please calculate the pH of the solution after the addition of 30 mls of 1 M HCl.

Since the pKa = 6.8 the pH = 7.4, the starting solution must be a buffer containing both conjugate acid and conjugate base forms of the compound in a total concentration of 1M. From the Henderson-Hasselbalch equation:

\[ \text{pH} = \text{pKa} + \log \left( \frac{[A^-]}{[HA]} \right) \]

\[ 7.4 = 6.8 + \log \left( \frac{[A^-]}{[HA]} \right) \]

\[ \log \left( \frac{[A^-]}{[HA]} \right) = 0.6 \]

\[ \frac{[A^-]}{[HA]} \approx 4 \]

\[ [A^-] = 4[HA] \]

and \([A^-] + [HA] = 1 \text{ M}\).

Therefore, \([HA] = 1 \text{ M/5} = 0.2 \text{ M} \) and \([A^-] = 0.8 \text{ M}\).

When you add 1 M HCl, it will convert (neutralize) an equimolar amount of the conjugate base form to the conjugate acid.

100 mls of 0.8 M conjugate base = 0.1 L x 0.8 moles/L = 0.08 moles of conjugate base present initially and conjugate acid = 0.1 L x 0.2 moles/liter = 0.02 moles. 30 mls of 1 M HCl contains 0.03 L x 1 mole/L = 0.03 moles. Subtracting this amount from the amount of conjugate base, 0.08 – 0.03 = 0.05 moles remain. And, adding to the conjugate acid, 0.02 moles + 0.03 moles = 0.05 moles are present after HCl addition. The total volume is 130 mls.

Now, \( \text{pH} = 6.8 + \log \left( \frac{0.05 \text{ moles}}{0.05 \text{ moles}} \right) \)

\[ \text{pH} = 6.8 + \log(1) = 6.8 \]

What would the pH of the solution be if 30 mls of 1 M NaOH were added instead of 1 M HCl?

30 mls of 1 M NaOH contains 0.03 L x 1 mole/L = 0.03 moles NaOH. It will completely neutralize the conjugate acid above. However, since there are only 0.02 moles of CA, the NaOH is in excess. Therefore, 0.03 moles NaOH – 0.02 moles CA = 0.01 moles NaOH remaining. Since it is a strong base, it will ionize completely and \([\text{OH}^-] = 0.01 \text{ moles/130 ml or 0.077 M}\). \([H^+] = 10^{-14}/0.077 \text{ M} = 1.3 \times 10^{-13} \text{ M} \) and \( \text{pH} = -\log(1.3 \times 10^{-13}) = 12.9 \)

7. a. (2 pts.) Calculate the standard free energy, \(\Delta G^\circ\), for the hydration of CO\(_2\) to form carbonic acid and for the dissociation of H\(_2\)CO\(_3\) to H\(^+\) and HCO\(_3^-\) described by the reactions and equilibrium constants given below:

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

\[ \text{K}_{\text{hydration}} = 5 \times 10^{-3} \text{M}^{-1} \]

\[ \text{K}_a_1 = 1.6 \times 10^{-4} \text{M} \]

The standard free energy, \(\Delta G^\circ\), can be calculated directly from the equilibrium constants given.

For hydration,

\[ \Delta G^\circ = -1.36 \text{Kcal/mole} \times \log K_{\text{hydration}} = -1.36 \text{Kcal/mole} \times \log 5 \times 10^{-3} = -1.36 \times -2.3 \text{Kcal/mole} = 3.13 \text{Kcal/mole} \]

For the first ionization,

\[ \Delta G^\circ = -1.36 \text{Kcal/mole} \times \log K_a_1 = -1.36 \text{Kcal/mole} \times \log 1.6 \times 10^{-4} = -1.36 \times -3.8 \text{Kcal/mole} = 5.17 \text{Kcal/mole} \]
b. (2 pts.) Using the same equilibrium constants, calculate the actual concentrations of CO$_2$, H$_2$CO$_3$ and HCO$_3^-$ that would be present in a solution of water maintained in a constant atmosphere containing a partial CO$_2$ pressure of 40 mm Hg which is sufficient to maintain a concentration of CO$_2$ + H$_2$CO$_3$ at 1.25 mM. For this calculation, assume that the ionization of H$_2$CO$_3$ has a negligible effect on its concentration. (Remember [H$_2$O] = 55.5M)

These are done using the equation for $K_{eq}$:

\[ K_{hydration} = \frac{[H_2CO_3]}{[CO_2][H_2O]} \text{ and } 5 \times 10^{-3} M^{-1} = \frac{[H_2CO_3]}{[CO_2][55.5M]} \text{ or } \frac{[H_2CO_3]}{[CO_2]} = 0.2775 \]

Since [H$_2$CO$_3$] + [CO$_2$] = 1.25 mM, [CO$_2$] + 0.2775[CO$_2$] = 1.25 mM or [CO$_2$] = 0.98 mM

And, [H$_2$CO$_3$] = 1.25 mM – 0.98 mM = 0.27 mM

Likewise, $K_a = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$ and 1.6 \times 10^{-4} M = \frac{[H^+][HCO_3^-]}{0.027 \times 10^{-3} M} \text{ But, } [H^+] \text{ must } = [HCO_3^-]

Therefore,

\[ 1.6 \times 10^{-4} M \times 0.27 \times 10^{-3} M = [HCO_3^-]^2 \text{ and } [HCO_3^-] = 2.08 \times 10^{-4} M \]

8. (1 pt.) For a chemical reaction that can take place either with or without an enzyme, please indicate in the space provided whether adding the enzyme would increase, decrease or cause no change in the

a. Activation energy of the reaction. Decrease

b. Initial velocity of the reaction. Increase

c. The free energy, $\Delta G$, of the reaction. No Change

d. The equilibrium constant for the reaction. No Change

9. (3 pts.) The turnover number for a 50 kDa (50,000 gms/mole) enzyme is known to be $10^5$ moles product produced per second per mole of enzyme. Please calculate the Km and Vmax for this enzyme from the following data set and compute the total amount of enzyme present in the assay used to generate these data. (Hint: Remember the definition of Km).

<table>
<thead>
<tr>
<th>Substrate Concentration (mM)</th>
<th>Initial Velocity (µMol/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>330</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>4</td>
<td>667</td>
</tr>
<tr>
<td>10</td>
<td>833</td>
</tr>
<tr>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

a. Km = 2 mM. If Vmax = 1,000 µMol/sec (see below), Km = [S] at Vmax/2 = 500 µMol/sec, the velocity when [S] = 2 mM. Both this and the answer for Vmax could also have been determined from 1/V and 1/[S] values by rise over range calculations.

b. Vmax There is no change in velocity between 100 and 1000 mM substrate. Therefore, Vmax = 1,000 µMol/sec

c. Total weight of enzyme in the assay =

The turnover number is given as $10^5$ moles product per sec per mole of enzyme. Vmax of the assays shown is $10^3$ µMol product per second. This is $10^3$ moles product/second. Since Velocity is linear with total amount of enzyme, the amount of enzyme in the assay would be: $10^3$ moles product/sec/$10^5$ moles product/second, mole enzyme $= 10^{-8}$ moles enzyme. Since enzyme MW = 50,000 gms per mole, amount of enzyme added was $5 \times 10^4$ gms/mol x $10^{-8}$ moles $= 5 \times 10^{-4}$ gms or 0.5 mg.
10. Both methanol and ethanol are metabolized by the liver enzyme alcohol dehydrogenase to yield aldehydes as shown below:

\[
\text{methanol} \quad \leftrightarrow \quad \text{formaldehyde} \quad \text{CH}_3\text{OH} \quad \leftrightarrow \quad \text{H} - \text{C} = \text{O} \\
\text{liver alcohol dehydrogenase} \\
\text{ethanol} \quad \leftrightarrow \quad \text{acetaldehyde} \quad \leftrightarrow \quad \text{acetic acid} \quad \text{CH}_3\text{CH}_2\text{OH} \quad \leftrightarrow \quad \text{CH}_3\text{C} = \text{H} \quad \leftrightarrow \quad \text{CH}_3\text{C} = \text{OH} \quad \rightarrow \quad \text{CO}_2 + \text{H}_2\text{O}
\]

While acetaldehyde produced from ethanol is converted ultimately to CO\(_2\) and H\(_2\)O, formaldehyde is not further degraded and is toxic. One of the primary treatments for methanol poisoning is oral administration of ethanol and monitoring the urine to determine when the concentration of excreted methanol has dropped to acceptable levels. The basis for this treatment comes from the behavior depicted below in the graph on the left of 1/V vs 1/[S] for production of formaldehyde as a function of methanol concentration (at saturating [NAD\(^+\)]) in the presence and absence of ethanol as indicated.

\[
\begin{align*}
\text{a. (2 pts.) From the graph on the left above, please calculate the values of Km and Vmax for methanol as a substrate for alcohol dehydrogenase.} \\
\text{Km} & = \text{determined from the X-intercept for the graph minus ethanol which is} \ -1/Km. \\
& = -1, mM^{-1} \text{ or } Km = 1 mM \\
\text{Vmax} & = \text{determined by the Y-intercept – ethanol.} \\
y \text{ intercept } = 1/V \max = .001/\text{umoles / min, mg enzyme} \text{ or } V \max = 1,000\mu\text{moles / min, mg enzyme}
\end{align*}
\]
b. (2 pts.) Please explain the type of effect seen with added ethanol and compute the relevant kinetic constants (i.e., $K_i$, $K_m$ etc) for ethanol’s action.

Since ethanol is a substrate, it binds competitively with methanol and thus acts as a competitive inhibitor. This is seen in the graphs since the curves ± ethanol cross on the y-axis indicating no difference in $V_{max}$ but intersect at different points on the x-axis giving apparent different $K_m$s indicative of competitive inhibition. The $K_i$ for ethanol, equal to $K_m$, can be calculated from the x-intercept $+ 1 \times 10^{-4} \text{M}$ ethanol which $= \frac{-1}{K_{mi}}$.

$$-\frac{1}{K_{mi}} = -0.5 \text{mM}^{-1} \text{ or } K_{mi} = 2 \text{ mM}$$

Since, $K_{mi} = K_m(1 + [I] / K_i)$  

$$2 \text{mM} = 1 \text{mM}(1 + 0.1 \text{mM} / K_i) \text{ and } 2 \text{mM}-1 \text{mM} = 0.1 \text{mM}^2 / K_i \text{ or } K_i = 0.1 \text{mM}$$

This answer also could have been inferred directly from the fact that $K_{mi} = \frac{1}{2} K_m$. Therefore, $[I] = K_i$.

c. (3 pts.) On the graph provided on the right, please draw the lines you would expect to obtain for $1/V_i$ vs $1/[S]$ for formaldehyde production with varying $[\text{NAD}^+] \pm 1 \times 10^{-4} \text{M}$ ethanol, if $[\text{methanol}] = 2 \text{ mM}$ and the $K_m$ for $\text{NAD}^+ = 0.1 \text{ mM}$. Please explain the basis for your answer briefly below.

Since $\text{NAD}^+$ is the second substrate, ethanol will be a non-competitive inhibitor reducing the apparent $V_{max}$, not $K_m$. Since $[\text{ethanol}]=K_i$, the apparent $V_{max}, V_{maxi}$, will be $V_{max}/2$ since

$$V_{maxi} = \frac{V_{max}}{(1 + [I] / K_i)} = \frac{V_{max}}{(1 + 1 \times 10^{-4} \text{M} / 1 \times 10^{-4} \text{M})} = \frac{V_{max}}{(1 + 1)} = V_{max}/2$$

11. (1 pts) Which of the terms in the Svedberg equation given below determines the direction of movement of a substance in the centrifuge and why?

$$S = \frac{\omega^2 r}{v} = \frac{M(1 - \frac{\rho_{solution}}{\rho_{particle}})}{f}$$

The density factors, $\rho_{solution}/\rho_{particle}$. If the density of the particle is less than 1 gm/cc (for example lipids), this term will be greater than 1 and the entire term becomes negative. This means material will float or move up in the centrifuge.
12. You have isolated a factor from amniotic fluid that stimulates the growth of pluripotent human embryonic stem cells. The following information has been obtained to characterize this material for possible use in human transplantation to treat numerous diseases.

a. The factor elutes from a gel permeation column with an apparent molecular weight of 850 daltons.

b. Treatment of this material with trypsin (Tp) or cyanogen bromide (CB) leads to complete loss of activity in concert with the generation of 2 smaller fragments in each case. These fragments were separated and subjected to three cycles of Edman degradation yielding the following information:

<table>
<thead>
<tr>
<th>Material</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB-1</td>
<td>Pth-Ala</td>
<td>Pth-Trp</td>
<td>Pth-Lys</td>
</tr>
<tr>
<td>CB-2</td>
<td>Pth-Pro</td>
<td>Pth-Arg</td>
<td>None detected</td>
</tr>
<tr>
<td>Tp-1</td>
<td>Pth-Ala</td>
<td>Pth-Trp</td>
<td>Pth-Lys</td>
</tr>
<tr>
<td>Tp-2</td>
<td>Pth-Gly</td>
<td>Pth-Asp</td>
<td>Pth-Met</td>
</tr>
</tbody>
</table>

1). (4 pts.) Please write the linear sequence of amino acids for this peptide deduced from these data in the space provided below:

H2N-Ala-Trp-Lys-Gly-Asp-Met-Pro-Arg-COOH

(To see how this sequence was deduced from the above data, see the attached page.)

2). (1 pt.) Circle the pH given below at which this peptide will move as indicated on electrophoresis. (Note: multiple answers are possible.)

<table>
<thead>
<tr>
<th>Movement</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toward the positive electrode</td>
<td>3.0</td>
</tr>
<tr>
<td>Toward the negative electrode</td>
<td>3.0</td>
</tr>
<tr>
<td>Toward neither electrode (no movement)</td>
<td>3.0</td>
</tr>
</tbody>
</table>

3). (3pts.) Please complete the diagram provided below by drawing in the structures of the amino acids side chains for tryptic peptide 2 from above and, where appropriate, the amide hydrogens. Also indicate with arrows the bonds in the amide backbone that are not free to rotate.
Deducing the sequence:

From the data given, the peptide is \(~\text{850 Da}\) and the average residue weight for each amino acid is given as \(105 \text{ Da}\) (Appendix). Therefore, the peptide must be 8 residues in length \((850/105 \approx 8)\).

Cyanogen bromide cleavage yields two peptides. CB-1 commences with the sequence Ala-Trp-Lys based on Edman degradation. CB-2 appears to have only 2 residues, Pro-Arg, and therefore most likely represents the C-terminus of the molecule. Otherwise, it would have had homoserine from the cleaved methionine.

Placing Pro-Arg at the C-terminus also agrees with the fact that only 2 peptides are obtained from trypsin cleavage even though the peptide has one residue each of Lys and of Arg. If both of these were internal, trypsin would give 3 peptides, not 2. Tp-1 gave the sequence Ala-Trp-Lys, the amino terminus of CB-1. Tp-2 gave the sequence Gly-Asp-Met that must be the C-terminus of CB-1.

Therefore, the only sequence compatible with these data is the one shown above.
4). (3 pt.) During the initial studies of the stem cell factor in amniotic fluid, it was observed that the activity was rapidly lost at room temperature. But, this loss was prevented by adding millimolar concentrations of diisopropylphosphofluoridate (DIFP). It was later found that the loss could also be prevented by adding the compound tosyl-L-phenylalanine chloromethylketone (TPCK). What protease is the most likely responsible for the destruction of the stem cell factor in amniotic fluid, which peptide bond(s) in the factor would it cleave and how do DIFP and TPCK block its action?

The loss is due to proteolysis. DIFP is a general inhibitor of serine hydrolases, including serine proteases, reacting with the active site serine. TPCK is a specific, affinity-based irreversible inhibitor of chymotrypsin reacting with the active site histidine. Hence, chymotrypsin is the responsible protease.

Chymotrypsin would cleave C-terminal to Trp at the Trp-Lys bond.

13. (10 pts.)

a.  

\[ \begin{align*} 
\text{R_1} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{O} \\
\text{CH}_2 & \\
\text{C} & \quad \text{H} \\
\end{align*} \]

d.  

\[ \begin{align*} 
\text{R_1} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{O} \\
\text{OH} & \quad \text{CH}_2 \\
\text{C} & \quad \text{H} \\
\end{align*} \]

b.  

\[ \begin{align*} 
\delta^- & \quad \delta^+ \\
\text{R_1} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{O} \\
\text{CH}_2 & \\
\text{C} & \quad \text{H} \\
\end{align*} \]

e.  

\[ \begin{align*} 
\delta^+ & \quad \delta^- \\
\text{R_1} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{OH} \\
\text{CH}_2 & \\
\text{C} & \quad \text{H} \\
\end{align*} \]

c.  

\[ \begin{align*} 
\text{R_1} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{OH} \\
\text{OH} & \quad \text{CH}_2 \\
\text{C} & \quad \text{H} \\
\end{align*} \]

The structures above represent intermediates and products formed during the cleavage of peptide bonds by the serine proteases. Select the letter of the structure that represents each of the following.
a. The transition state for the cleavage of the acyl enzyme intermediate ___e__.

b. The acyl enzyme intermediate ___a__.

c. The initial enzyme substrate complex ___d__.

d. The transition state leading to the formation of the acyl enzyme ___b__.

e. The final products of the reaction ___c__.

Also answer the following questions about the serine proteases in the space provided.

f. Indicate which amino acids below could be the side chain indicated as R1 in the diagrams above for cleavage of the substrate by chymotrypsin ___1, 2, 8___ or trypsin ___3, 4___ . Multiple answers are possible.

1) Tyrosine     4) Arginine     7) Histidine
2) Tryptophan   5) Glycine      8) Phenylalanine
3) Lysine       6) Aspartic Acid 9) Serine

g. Which amino acids in this list form the catalytic triad ___6, 7, 9___.

14. (4 pts.) Using the cartoon of ribonuclease shown below, draw arrows to and label the four basic types of secondary structures of which proteins are composed.

15. (5 pts.) Below please list the five types of non-covalent interactions that dictate folding of ribonuclease and other proteins and discuss briefly their role in stabilization of these secondary structures and in providing energy for the folding process.

   a. Hydrophobic. The hydrophobic effect results from the increased entropy as H₂O molecules are released from cage structures as aliphatic side chains are buried away from solvent. This is the primary source of free energy that drives protein folding and protein-protein interaction.
b. **van der Waals interactions.** This is an electric dipole interaction induced by electron cloud distortion as atoms approach overlap distances. Important in the formation of $\alpha$-helix and in dictating precise folding on the protein interior and providing additional energy for folding. Also important for proper alignment of amino acid side chains for example in the case of the leucine zipper structure.

c. **H-bonds.** Dipole-dipole interaction between a partial positively charged proton attached to an electronegative atom (N, O) and an electronegative atom with partial negative charge. These interactions stabilize all elements of secondary structure, $\alpha$-helix, $\beta$-sheet and $\beta$-bend. However, they provide no net energy for folding since these groups also H-bond with water.

d. **Salt bridges (electrostatic interactions).**
True ionic interactions between formal + and – charges on surface amino acid side chains as well as with $\alpha$-NH$_3^+$ and $\alpha$-COO$^-$ groups. Provide for stabilization and local structure alignment. No energy for folding as these charges will also interact equally well with solvent salt ions.

e. **Aromatic stacking.**
Stacking of aromatic rings due to ring current effects. Provides only localized precise alignment.

16. (1 pt.) Ribonuclease and many other proteins designed for extracellular environments have covalent _disulfide_ bonds in addition to the amide backbone that form before or after (circle one) the protein is completely folded.

17. At rest, the arterial blood of a cross-country runner has a pH of 7.4, the concentrations of bicarbonate and carbonic acid in arterial plasma are 25 mM and 1.25 mM, respectively, and the partial pressure of alveolar CO$_2$ is 40 mm Hg.

   a. (2 pts.) After a short jog to warm up prior to a 25 kilometer (15.5 mile) race, the concentration of lactic acid in this runner's blood increases from near zero at rest to 4 mM. Calculate the new partial pressure of CO$_2$ necessary to maintain the runner's blood at pH 7.4.

   Lactic acid is a stronger acid than carbonic acid. Therefore, the entire 4 mM would convert an equivalent amount of HCO$_3^-$ to H$_2$CO$_3$ reducing the concentration from 25 mM to 21 mM. However, pH stays at 7.4. Therefore, H$_2$CO$_3$ must also be reduced as calculated from the Henderson-Hasselbalch equation.

   $pH = pKa + \log \frac{[HCO_3^-]}{[H_2CO_3]}$ or 7.4 = 6.1 + $\log \frac{21\text{mM}}{[H_2CO_3]}$ and $\frac{21\text{mM}}{[H_2CO_3]} = 20$ or $[H_2CO_3] = 21/20 \text{mM} = 1.05\text{mM}$

   The partial pressure of CO$_2$ that gives this concentration is $(1.05\text{mM}/1.25\text{mM}) \times 40 \text{mm Hg} = 33.6 \text{mm}$
b. (2 pt) At the end of the 25 K race, the concentration of lactic acid in the runner's blood is now 15 mM and the partial pressure of CO2 in the runner's alveoli is now 25 mm Hg. Calculate the pH in the runner's arterial blood.

If \( PCO_2 = 25 \text{ mm Hg} \), \( [H_2CO_3+CO_2] = 25\text{ mm Mn} \times 1.25 \text{ mM} = 0.78 \text{ mM} \)
And, \( [HCO_3^-] = 25 \text{ mM} - 15 \text{ mM} = 10 \text{ mM} \)

\[
pH = 6.1 + \log \left( \frac{10_{\text{M}}}{0.78_{\text{mm}} \text{M}} \right) = 6.1 + 1.1 = 7.2
\]

c. (4 pts) Using the graph at the right, sketch the respective oxygenation curves for hemoglobin in the erythrocyte under the conditions set forth in a. and b. above labeling each one appropriately. Briefly explain below why these curves are different.

The oxygenation curve for HbA at pH 7.2 is shifted to slightly higher PO2 (lower affinity) compared to that at 7.4 due to the Bohr effect. This results from the making and breaking of salt bridges, especially involving His residues that help stabilize the deoxy conformation.

d. (2 pts). Also include and label in the graph in c above the oxygenation curve expected for the runner at rest if they had a genetic defect allowing expression of only fetal hemoglobin and explain below the basis for its difference from that for HbA under the same conditions.

HbF has a higher affinity for O2 than HbA because it has a lower affinity for the allosteric regulator 2,3-BPG. BPG acts as an allosteric inhibitor of O2 binding because it binds preferentially to the deoxy conformation. The \( \gamma \)-chains that replace the \( \beta \)-chains in HbF lack a critical His residue involved in BPG binding, hence the lower affinity.

18. (1 pt.) In hemoglobin and myoglobin, the heme Fe\(^{2+}\) is coordinated to the four heme pyrrole nitrogens and the imidazole nitrogen of the distal / proximal (circle one) histidine while the distal / proximal (circle one) histidine alters the geometry of carbon monoxide binding to the heme Fe\(^{2+}\) decreasing its binding affinity substantially.
Formulas:

\[ \Delta G' = \Delta G^{\circ} + R T \ln \left[ \frac{[C][D]}{[A][B]} \right] \]

where

- \( R \) = the gas constant
- \( T \) = Absolute Temp., °K
- \( RT \ln = 1.36 \log_{10} \) at 25°C

\[ K_{m}' = K_m \left(1 + \frac{[I]}{K_i} \right) \]

and

\[ V_{\text{max}}' = \frac{V_{\text{max}}}{(1 + \frac{[I]}{K_i})} \]

For CO₂, the solubility constant \( \alpha = 0.03125 \)

Average amino acid residue weight = 105 Da