Recitation #2

Contact Information
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Recitation: Friday, 3-4pm, 2-132
Office Hours: Friday, 4-5pm, 2-132

Unit 1 Schedule
Recitation/Exam Date Lectures covered
Recitation #2 Friday, February 27 4, 5, 6
Recitation #3 Tuesday, March 2, 5pm, 2-132 7, 8, 9
Exam 1 Review Thursday, March 4, 7pm, 35-225 1-9
Exam 1 Monday, March 8, 9:30-11am, Walker 1-9

Recitation Overview

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<td>1. The Genetic Code</td>
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<tr>
<td>3. pH, pKa, and pI</td>
<td>4, 5, 6</td>
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<td>4. Amino Acids and Polypeptides</td>
<td>6</td>
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<tr>
<td>5. Henderson-Hasselbach Equation, Buffers, Titration Curves</td>
<td>7, 8, 9</td>
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Problems

1. Assuming random translation starts, what products would you expect upon addition of poly-(CGA) RNA to a cell-free protein synthesis system? Propose a column-based strategy to separate these products.

2. (2001 Exam 1 Question 5, 15 points)
   Consider the biological synthesis of protein using the amino acid Ala. If the two oxygen atoms of Ala were replaced with heavy isotopes of oxygen so that we could follow the fate of these two oxygens during protein synthesis, in which residue/molecule(s) would these two oxygens reside after translation is complete? Draw the structure of the residue/molecule(s), and circle the heavy-oxygen atoms.
3. (2002 Exam 2 Question 5, 20 points)
An mRNA molecule encoding a protein of 100 amino acids is translated in a cell-free protein synthesis system in which ATP, GTP, and amino acids are added.
(a) Suppose that for every protein molecule produced, an average of 272 GTP molecules are hydrolyzed to GDP. Provide a detailed accounting for how this number of GTP molecules are likely to have been consumed (i.e., for each part of the translation process that uses GTP, state the number of GTP molecules used, and provide a rationale for choosing that number).

(b) Suppose that for every protein molecule produced, an average of 117 ATP molecules are hydrolyzed to AMP. Account for how this number of ATP molecules would be consumed.

4. Given the polypeptide Ala-Asp-Val-Lys
(a) Determine the net charge at each of the pKₐ values of the ionizable groups and at pH 1 and pH 12. (pKₐ values - terminal amino group: 8.1, terminal carboxyl group: 3.1, aspartate: 4.3, lysine: 10.8).

<table>
<thead>
<tr>
<th>pH</th>
<th>Terminal Amino</th>
<th>Terminal Carboxy</th>
<th>Aspartate</th>
<th>Lysine</th>
<th>Net Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3.1</td>
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<tr>
<td>4.3</td>
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<tr>
<td>8.1</td>
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<tr>
<td>10.8</td>
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</tr>
<tr>
<td>12</td>
<td></td>
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</tbody>
</table>

(b) Over what range of pH would the net charge of the peptide = 0?
5. (1995 Exam 1 Question 1 modified)
Aspirin (acetyl salicylic acid) has a carboxylate with a $pK_a$ of 3.5. In order to enter the bloodstream, aspirin must pass through the membrane lining the stomach (pH ~ 1) and small intestine (pH ~ 6). In general, electrically neutral molecules pass through a membrane more easily than charged molecules. Would you expect more aspirin to be absorbed in the stomach or small intestine? Why?

![Aspirin (acetyl salicylic acid)](image)

6. (2001 Exam 1 Question 6, 25 pts)
Consider the use of isoelectric focusing to separate the three polypeptides, A, B, and C.
A = AlaGlyHisProGlnThrVal
B = IleLeuCysTyrAspGluAla
C = LysArgPro
(a) (3 pts) Using the table of amino acid abbreviations, write the sequence of these three polypeptides using the one-letter symbols
(b) (12 pts) Using the table of $pK_a$ values, predict which residues of these polypeptides will be predominantly charged when these polypeptides are focused at their respective isoelectric points; circle these residues in the three sequences that you have written in part (a). For the residues that you circled, indicate whether the charge would be positive or negative.
(c) (6 pts) Sketch a simple diagram of the separation of these three polypeptides by isoelectric focusing. Indicate which part of the gel is high pH, and which is low pH. Indicate the polarity of the electric field. Indicate the relative positions of the three polypeptides. Please do not calculate the precise pI’s of these polypeptides, show only their positions relative to the pH gradient, the electric field, and each other.

(d) (6 pts) (Try after class) Draw the chemical structure of the predominant species of peptide C when it is focused at its isoelectric point.

7. (2001 Exam 2 Question 1, 10 points)
   Sketch the titration curve for glutamic acid and indicate (with structural formulas) the charged species obtained during the phases of the titration. Be sure to label the axes properly. [The pKₐ values of glutamic acid are 2.2, 4.3, and 9.7]
8. (2002 Exam 1 Question 3, 10 points)

The figure below shows the titration curve of one of the common amino acids.

(a) (2 pts) What is the amino acid?

(b) (3 pts) What is going on at points A, C, and E?

(c) (3 pts) What is the pI (isoelectric point) of the amino acid?

(d) (2 pts) What is the net charge at points B and D?

9. Calculate the pH of a solution containing 0.75 M lactic acid \( (K_a = 1.4 \times 10^{-4}) \) and 0.25 M sodium lactate. Lactic acid \( (\text{HC}_3\text{H}_5\text{O}_3) \) is a common constituent of biologic systems. For example, it is found in milk and is present in human muscle tissue during exertion.

**Practice Problems**

10. (1999 Exam 1 Question 6, 10 points)

Propose two short DNA oligonucleotides (12 nucleotides each) that you would synthesize if you wanted to use the Khorana method for making polymers that could be used in a cell-free transcription and translation extract to generate poly-Ile and poly-Asn.
11. (1996 Exam 1 Question 4, 10 points)

tRNA synthetases “charges” tRNA molecules in a two-step reaction. Suppose that you carried out both steps in the presence of radioactive ATP ($^{32}$P incorporated at all phosphorous atoms in ATP). Where would you expect the radioactivity to be located after the charging was complete? Explain briefly.
Amino Acids and Polypeptides
Victor Sai, 7.05 Spring 2004

General Structure of a polypeptide

Chiral centers: α carbons (except that of glycine); both Isoleucine and Threonine have an extra chiral center in their side chains

Rotatable bonds: all bonds except those in peptide units, in rings, or of carboxy groups

Note: Side chains alternate (trans configuration)

Side Chains of Amino Acids

Hydrocarbons

Hydrophobic SIDE CHAINS

<table>
<thead>
<tr>
<th>Glycine</th>
<th>Alanine</th>
<th>Valine</th>
<th>Leucine</th>
<th>Isoleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gly, G)</td>
<td>(Ala, A)</td>
<td>(Val, V)</td>
<td>(Leu, L)</td>
<td>(Ile, I)</td>
</tr>
</tbody>
</table>

Imino Acid

(commonly cis to neighboring residues)

 Cannot rotate; hinders polypeptide conformations

Proline

(Pro, P) Full Structure (not remainder group)

Sulfur-containing

Cysteine

(Cys, C)

Methionine

(Met, M)

Aromatic

Phenylalanine

(Tyr, Y)

Tryptophan

(Trp, W)
### Hydrophilic Side Chains

- **Alcohols**
  - (polar)
  - Serine (Ser, S)
  - Threonine (Thr, T)

### Charged Amino Acids

#### Cations
- **(basic)**
  - Lysine (Lys, K) \( pK_a = 10.8 \)
  - Arginine (Arg, R) \( pK_a = 12.5 \)
  - Histidine (His, H) \( pK_a = 6.0 \)

#### Anions
- **(acidic)**
  - Aspartate (Asp, D) \( pK_a = 4.3 \)
  - Glutamate (Glu, E) \( pK_a = 3.9 \)
**pH, pK_a, and pI**

**pH**: concentration of protons in an environment (higher pH: fewer protons)

**pK_a**: ability of an acid to keep its proton (higher pK_a: greater ability to keep its proton)

**pI**: pH at which net charge is 0

<table>
<thead>
<tr>
<th>pH</th>
<th>Protonated?</th>
<th>Charge if acidic</th>
<th>Charge if basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH &lt; pK_a</td>
<td>Protonated</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>pK_a</td>
<td>½ protonated, ½ deprotonated</td>
<td>-0.5</td>
<td>+0.5</td>
</tr>
<tr>
<td>pH &gt; pK_a</td>
<td>Deprotonated</td>
<td>-1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Relationship between pH and pI:**

<table>
<thead>
<tr>
<th>pH</th>
<th>Net Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH &lt; pI</td>
<td>+</td>
</tr>
<tr>
<td>pI</td>
<td>0</td>
</tr>
<tr>
<td>pH &gt; pI</td>
<td>-</td>
</tr>
</tbody>
</table>

**Protein Analysis and Purification**

1) **Protein Analysis**: Determining what proteins are present
   a) *SDS-PAGE*: Separation by Molecular Weight
      - charge provided by SDS – 1 SDS/ 2 a.a.
      - drag introduced by polyacrylamide
      - disulfide bonds reduced by β-mercaptoethanol
      - detection by Coomassie blue or silver stain
   b) *Western Blot*: If antibody to the protein is available, a Western Blot tells whether the protein of interest is present in a sample
   c) *Isoelectric Focusing (IEF)*: Separation by pI.
      - pH gradient set up by pre-running the gel with ampholytes
   d) *2D gel*: First perform IEF, then do SDS-PAGE

2) **Protein Purification**: Purifying a protein for further studies
   a) *Dialysis*: Separation by size, small molecules go through membrane
   b) *Gel-filtration*: Better way of separating by size, larger molecules elute first
   c) *Salting Out*: High salt concentration leads to less solubility. Different salt concentration needed for different proteins.
   d) *Ion Exchange*: Separation by charge
      - To select for negative proteins, use a DEAE column (aka anion-exchange column, contains positively-charged beads that will attract negatively-charged proteins and repel positively-charged proteins)
      - To select for positive proteins, use a CM column (aka cation-exchange column, contains negatively-charged beads that will attract positively-charged proteins and repel negatively-charged proteins)
   e) *Affinity Chromatography*: Separation by affinity for a specific chemical group.
      - Elute with substance that competes with the binding of the beads to the protein (e.g., Elute (His)_6-tagged protein with imidazole, functional group of Histidine; Elute Maltose Binding Protein with maltose)
Translation

- tRNA charging by aminoacyl-tRNA Synthetases:
  Overall reaction: amino acid + ATP + tRNA $\rightarrow$ aminoacyl-tRNA + AMP + PP_i
  At least one aminoacyl tRNA synthetase exists for each amino acid.
  1) First step: Amino acids are activated by ATP
     amino acid + ATP $\rightarrow$ aminoacyl-AMP
  2) Second step: Amino acids are attached to tRNA
     aminoacyl-AMP + tRNA for the amino acid $\rightarrow$ 2’ or 3’ aminoacyl-tRNA + AMP

- Fidelity of charging by editing or proofreading mechanisms:
  1) Consider the Ile tRNA synthetase mischarging of valine to get Val-tRNAIle: Isoleucine binding in the first step is favored by only ~100-fold over valine. However, addition of tRNAIle leads to 100% hydrolysis of the incorrectly activated valyl-AMP intermediate.
  2) Hydrolytic editing occurs at a second active site. The "double sieve" sorts correct and incorrect charging. Although both the correct and incorrect amino acid might fit into the first sieve, which detects amino acids by their size and shape and adenylates the amino acid, the incorrect amino acid will be hydrolyzed (destroyed) after fitting into the second sieve, which is a hydrolysis pocket too small for the correct amino acid to fit.
  Note: Not all of the synthetases use editing steps, for example, Tyr is sufficiently different from the other 19 amino acids (size and chemical characteristics) that substrate specificity alone ensures accurate charging.

- Codon-Anticodon Interactions:
  1) Charged tRNAs are selected by ribosomes solely through codon-anticodon interactions.
  2) Due to the degeneracy of the code, the third position codon-anticodon pairing is unstable and therefore also called a "wobble" base pair.

- Ribosome: protein-synthesizing machine.
  The ribosomal subunits and their RNA components are named for their sedimentation coefficients. The E. coli ribosome is 70S, which is composed of a 50S and a 30S subunit, and each contains a number of proteins and RNA.
  Eukaryotic ribosomes: Similar but the RNA's are larger and the proteins are more numerous.

- Protein (polypeptide) Synthesis:
  - Translation occurs on polyribosomes. The polypeptide grows stepwise as a ribosome bound, peptidyl-tRNA.
  - Discrete sites on both ribosomal subunits accommodate steps in elongation.
    a) The P site binds the peptidyl-tRNA
    b) The A site binds the aminoacyl-tRNA corresponding to the next amino acid.
    c) The E site is the exit site for the tRNA that was previously in the P site.
  - The path of a tRNA through the ribosome is: A site $\rightarrow$ P site $\rightarrow$ E site.