Paper Chromatography of Amino Acids

Prior to lab you should:

- Make sure you:
  - know the relationship between **proteins** and **amino acids**
  - know the "**conserved region**" of the amino acids
  - know what an **–R group** is
  - understand the basics of **paper chromatography**
    - How is it performed?
    - What causes hydrophilic molecules to migrate slower than hydrophobic molecules?
    - what makes molecules **hydrophobic/hydrophilic?**
  - are able to calculate an **R_f factor**

I. Objectives:

- **To determine the relationship between the chromatographic properties and the chemical structures of amino acids**
- **Use paper chromatography to investigate the chemical structure of an unknown amino acid.**

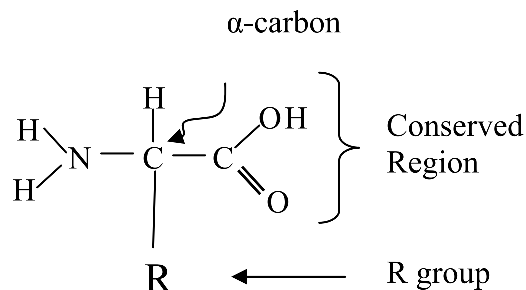
II. Background:

Amino Acids

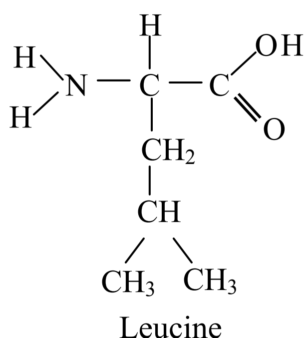
The thousands of different cellular proteins carry out distinct biological processes. The specific process mediated by a protein is dependent on the protein's three dimensional shape. Ultimately, this three dimensional shape is dependent on the chemical structure of the protein.

Proteins consist of long polymers called polypeptides, strings amino acids linked together by peptide bonds. All polypeptides are composed of the same set of twenty amino acids. Different proteins vary in the order and number of amino acids in their polypeptide chains.

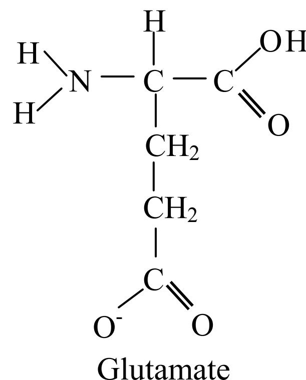
All twenty amino acids share a common structure called the "conserved region" of the amino acid. This conserved region consists of a central carbon called the α-carbon. This α-carbon is linked to a carboxyl group, an amino group and a hydrogen atom. These groups along with the α-carbon make up the "conserved region". All twenty amino acids have this structure. The α-carbon is also attached to a variable structure called the R group. The R group is what differs among the twenty amino acids.



Consider the two amino acids drawn below. Can you identify the conserved region and the R group of the amino acids? Based on the chemical structure of the R group, can you predict which amino acids would be more hydrophilic and more hydrophobic?



Leucine



Glutamate

Paper Chromatography

Chromatography is an analytical tool for distinguishing different biomolecule based on their chemical properties. One of the oldest and most reliable forms of chromatograpy is paper chromatography. In this assay, a biomolecule (or mixture of biomolecules) is spotted on a piece of filter paper. The filter paper is composed mostly of cellulose and is very hydrophilic. Next a hydrophobic organic solvent is drawn up the paper by capillary action. As the solvent moves over the location of the biomolecule, the biomolecule begins to move up the paper. The rate at which the biomolecule moves up the paper is related to its relative affinity for the paper (which is hydrophilic) and the solvent (which is hydrophobic). Hydrophobic molecules will move faster because they are more attracted to the hydrophobic solvent than the hydrophilic paper. On the other hand, hydrophilic molecules will move slower because they are attracted more to the paper than the hydrophobic solvent.

Paper chromatography is especially useful in characterizing amino acids. The different amino acids move at differing rates on the paper because of differences in their R groups.

The rate of movement of a biomolecule during paper chromatography is reported as its relative mobility (R_f). R_f is simply the distance the biomolecule moved through the filter paper divided by the distance the solvent moved through the paper. See the appendix for more details on how to measure R_f.

III. Materials:

The Following materials should be available at your lab station:

5 known amino acids (2mg/ml in 10% isopropanol: 0.1M HCl)
    Leu, Ala, Phe, Asp, Ser
1 unknown amino acid (2mg/ml in 10% isopropanol: 0.1M HCl)
Gloves
Whatman chromatography paper (21 X 21cm)
Pencils and Rulers
p20 with small pipette tips
Stapler
Waste beaker

IV. Procedure:

1. The chromatography chambers (located in the hood) will be set up prior to lab by the lab instructor to allow them to "equilibrate".

2. **WEARING GLOVES**, obtain a clean piece of filter paper.
   The filter paper should never be handled by bare hands since the skin's oils show up on the developed chromatogram

3. Using a pencil and ruler, draw a line approximately 3 cm from the bottom of the paper. Then every 3 cm on the line draw a circle 2 mm in diameter (there should be six circles spread evenly across the line (figure 1) Label each circle in pencil (Leu, Ala, Phe, Asp, Ser, Unkn)

4. Start with the 1st amino acid sample. Pipette 2µl on to the appropriate circle **Use a new pipette tip** to pipette the 2nd amino acid. Continue for all samples. Then:
   - Let all samples dry
   - **Repeat 4x** so that each circle has 10µl of the appropriate sample

5. When the last sample is dry, roll the paper into a cylinder and staple so that the edges do not touch (figure 2).

6. Stand the cylinder in a chromatography chamber (under the hood). Cap the chamber and allow the chromatogram to develop for **60-90 minutes** or until the solvent line is within an inch from the top of the paper.

7. Under the hood, remove the paper from the jar and immediately mark the solvent front line in pencil. Allow the paper to thoroughly dry by hanging it on the wire in the hood.

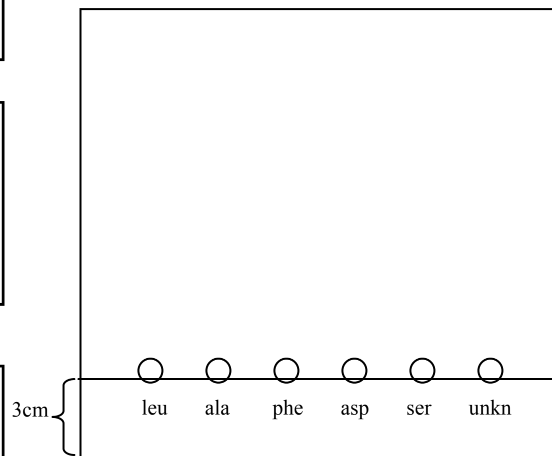


Figure 1: How to set up your chromatogram. Make sure the line is 3cm from the bottom and the samples are at least 1 inch from the edge.

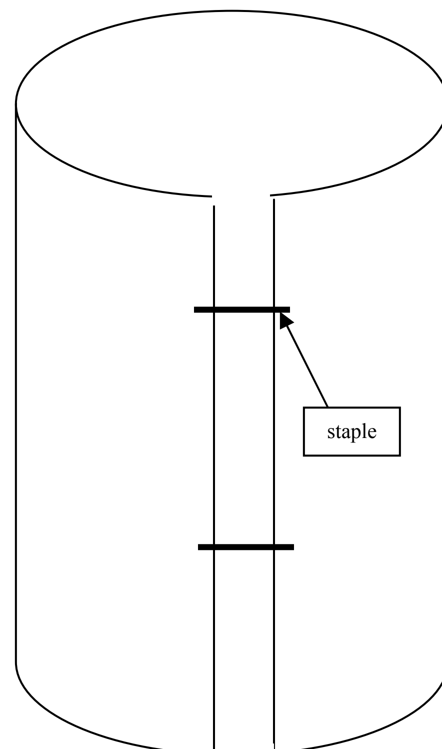


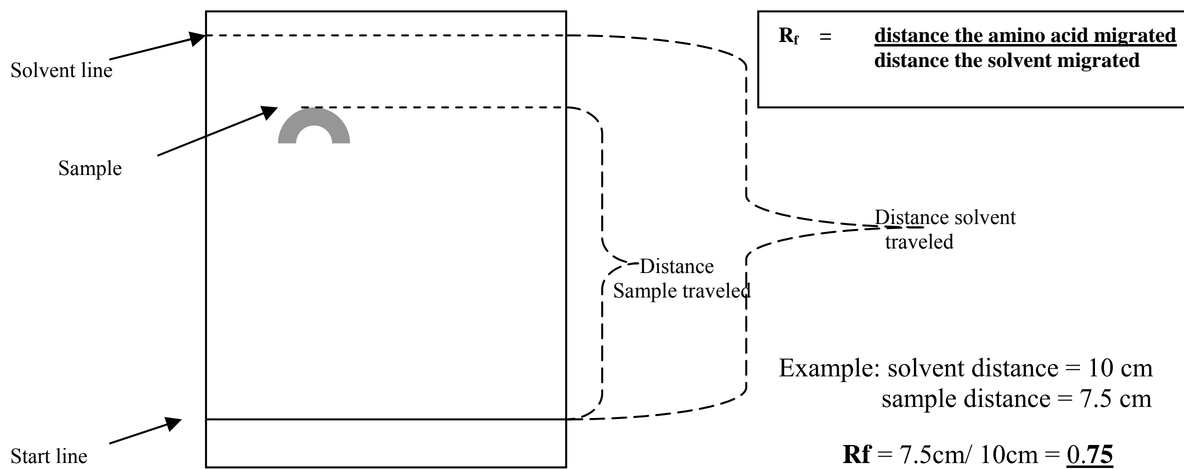
Figure 2: Stapling the chromatogram .

8. Your lab instructor will spray your paper with Ninhydrin developer, which is used to detect the location of amino acids. The paper will then be placed in a drying oven at about 100 C for 3-4 minutes to allow the color to develop.

9. Measure the distance the solvent migrated and the distance each of the amino acids migrated. Record the measurements on the attached data sheet. Calculate the relative mobility (R_f) for each amino acid.

$$R_f = \frac{\text{distance the amino acid migrated}}{\text{distance the solvent migrated}}$$

B. Determining the R_f value



V. Short Report

Name: ___________________________
Due at next laboratory session

1. Data Collection Sheet

Distance Solvent Migrated ______________

Amino Acid	Distance Migrated	Rf Value
Leucine		
Alanine		
Phenylalanine		
Aspartic Acid		
Serine		
Unknown		

2. Based on the chemical **<u>structures in your textbook</u>** rank the five known amino acids from most hydrophilic to most hydrophobic. Write the names of the 5 amino acids in the table below from most hydrophilic to most hydrophobic. (**Hint:** The more carbon and hydrogen in the structure the more hydrophobic, polar groups reduce hydrophobicity and ionizable groups reduce hydrophicity more than polar groups.)

Most Hydrophilic
Most Hydrophobic

3. Compare the R_f values of Phe and Ser. Is this consistent with their chemical structures? Explain.

4. Assume that the unknown amino acid that you analyzed was not one of the 5 known amino acids on your chromatogram (Leu, Ala, Phe, Asp, Ser), but instead was one of the other 15 amino acids. Based on its R_f , what is the most likely identity of your unknown. Justify your conclusion.

5. If you boil an protein in hydrochloric acid it will break down to its constituent amino acids. Assume that you had done this to a typical protein and then had spotted these amino acids to a spot on the chromatograph. What pattern would you expect to see at the end of the experiment? Explain your prediction.