Thin-Layer Chromatography of Amino Acids
HASPI Medical Biology Lab 15b

Background

Macromolecules
There are four major types of biological macromolecules that make up the human body: nucleic acids (DNA & RNA), carbohydrates, proteins, and fats. The following diagram summarizes the polymers and monomers of the four major macromolecules.

Amino Acids
There are 20 essential amino acids used to form a protein. Each amino acid has a slightly different chemical structure. Proteins are responsible for many important functions in the body and the table below summarizes only a few of these functions.

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein Class</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defense</td>
<td>Antigens</td>
<td>Mark foreign proteins, like those on bacteria, for the immune system to destroy</td>
</tr>
<tr>
<td>Transport</td>
<td>Membrane</td>
<td>Carry glucose into cells to be converted into energy</td>
</tr>
<tr>
<td>Increase chemical reactions</td>
<td>Enzymes</td>
<td>Break down carbohydrates, proteins, and fats in the body during digestion</td>
</tr>
<tr>
<td>Support</td>
<td>Fibers</td>
<td>Form cartilage, hair, and nails</td>
</tr>
<tr>
<td>Regulation</td>
<td>Binding</td>
<td>Store calcium in the bones</td>
</tr>
<tr>
<td>Movement</td>
<td>Muscle</td>
<td>Contract muscle fibers</td>
</tr>
<tr>
<td>Storage</td>
<td>Hormones</td>
<td>Control the amount of glucose in the blood</td>
</tr>
</tbody>
</table>

The shape and function of a protein depends on the number and order of the amino acids in the protein, just as the combination of letters in the alphabet can create a different word, sentence, or paragraph.
Thin-Layer Chromatography

Chromatography is a process used to separate a mixture of different substances back into their individual forms. For example, if you mixed red, yellow, green, and blue food coloring together chromatography could be used to separate them back into their individual colors. There are many different types of chromatography depending on the substances that need to be separated. The most commonly used methods include paper chromatography, gas chromatography, liquid chromatography, and thin-layer chromatography.

Thin Layer Chromatography (TLC) is used to separate solids from a liquid. The most common use is to separate amino acids from a liquid and each other. A spot of the sample is placed on a sheet of glass treated with an absorbent substance. The glass is then placed in a solvent that will travel up the absorbent surface and cause the solid to move out of the liquid with it. Different solids will move different distances on the sheet, but the distance will remain constant no matter how many times chromatography is done. This distance is calculated into an amount called the Rf value, which can be used to determine the identity of the substance.

**Rf values**

The distance a substance travels related to the distance the solvent travels is called the Rf value. The Rf value can be calculated by measuring the distance of the substance from its starting point in millimeters, as well as the distance the solvent traveled from its starting point in millimeters, then dividing the substance distance by the solvent distance. The equation is:

\[
Rf = \frac{\text{Substance Distance}}{\text{Solvent Distance}}
\]

**Example:**

\[
Rf = \frac{32 \text{ mm}}{66 \text{ mm}} = 0.48
\]

It does not matter if the solvent moves 10 mm or 100 mm, the Rf value of a substance will remain the same. For example the average Rf value for a dye called “methylen blue” is 0.50. So, that means if the substance moved 5 mm, the solvent moved 10 mm. It also means if the substance moved 50 mm, the solvent moved 100 mm.

**Phenylketonuria**

Phenylalanine is an amino acid. A genetic disorder in which the body is not able to break down or use phenylalanine is called phenylketonuria or PKU. If an individual with PKU consumes too much phenylalanine he or she can suffer from brain damage that may cause mental retardation, seizures, and growth delays.

Phenylalanine is found in beef, milk, eggs, and artificial sweeteners, so individuals with PKU have to be very careful about what they eat. Any product containing phenylalanine must have a warning to help those with PKU avoid those products.

Purpose: The goal of this lab will be to use thin-layer chromatography to determine whether a patient who has been diagnosed with PKU may have accidentally consumed phenylalanine.

Scenario:
A 15-year-old male patient that has been diagnosed with PKU was recently brought into the ER at HASPI Hope Hospital suffering from screaming fits and seizures. His PKU was not recognized until he was 2 years old and much of the damage to his brain up to that point is irreversible. Since his diagnosis, the parents have been extremely careful with his diet to make sure he does not consume any phenylalanine. In order to determine whether his current seizures are the result of him accidentally consuming phenylalanine, or due to something else, testing needs to be performed.

He was eating some pork sausage and drinking a mixed juice about an hour before his seizures started. You have been asked to determine if either of these items he consumed may have had untagged phenylalanine in them.

Thin-layer chromatography will be used to compare a prepared phenylalanine solution to a solution of the mixed juice (Amino Acid Solution A) and a solution of the pork sausage grease (Amino Acid Solution B).

1. Obtain a TLC plate. Avoid touching the coated surface, since fingerprints can leave significant quantities of protein and therefore ruin them.
2. Draw a light pencil line about 1 cm from the bottom of the plate.
3. Evenly space 3 marks across the pencil line. These will be the places where you spot your amino acid samples on the plate. Label each mark at the bottom of the plate—P for the phenylalanine solution, A for amino acid solution A, and B for amino acid solution B—so that you can identify them later.
4. Take your plate to the phenylalanine solution. Place the tip of the capillary tube into the phenylalanine solution and place the tip on the first mark labeled P. The spot should be small: more is not better! This step is very sensitive and large spots lead to incorrect results.
5. Allow the sheet to dry for 15-20 seconds and spot-mark P with the phenylalanine solution capillary tube again.
6. Spot a small sample of amino acid solution onto each mark with a capillary tube. Spots should be small: more is not better here! The method is very sensitive. Large spots lead to imprecise results.
7. Measure out 2 mL of chromatography solvent and CAREFULLY pour it into the bottom of the hinge-topped vial. Try to not allow the solvent to run down the sides of the vial.
8. Place the end of the TLC plate with the P, A, and B spots into the hinge-topped vial. MAKE SURE THAT THE LINE AND SPOTS DO NOT TOUCH THE SOLVENT!
9. Close the top on the vial to prevent the solvent from evaporating.
10. Watch the chromatography plate closely as the solvent moves up the strip. If the solvent reaches the very top before it is stopped, the experiment is invalid because an Rf value cannot be determined.

11. You will not see the amino acids moving on the plate since they are colorless. We will use a UV black light to see them.

12. Allow the solvent to move until it gets approximately 1 cm from the top of the plate. Remove the plate and place it flat on the paper towel to dry. If there is not enough time to allow the solvent to move that far, remove the plate regardless of the distance traveled since Rf values can still be calculated.

13. Make a mark in pencil on the strip where the solvent was stopped.

14. Pour any leftover solvent in the vial back into the chromatography solvent bottle.

**Analysis**

**Analyzing the TLC Plate**

1. Place the TLC plate under a black light source. The black light source should make any amino acids visible. Using the pencil, make a mark on the plate where the amino acids present in any of the samples stopped moving.

2. Measure the distance in millimeters from the start (pencil mark at the bottom of the strip) to the end of the solvent movement. Record that distance below.

3. Measure the distance in millimeters from the starting point to where each of the amino acids stopped. Record the distances in Data Table 1.

4. Calculate the Rf values for each amino acid. Refer to the background information for details and examples.

5. Compare your calculated Rf values to Data Table 2 to determine the possible amino acids found in each solution. Complete the Analysis Section.

**Data Table 1**

<table>
<thead>
<tr>
<th>Phenylalanine Solution</th>
<th>Amino Acid Solution A (Mixed Juice)</th>
<th>Amino Acid Solution B (Sausage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance Moved</td>
<td>Rf Value</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data Table 2 – Average Rf Values of Amino Acids**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Rf Range</th>
<th>Amino Acid</th>
<th>Rf Range</th>
<th>Amino Acid</th>
<th>Rf Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.30-0.46</td>
<td>Histidine</td>
<td>0.12-0.32</td>
<td>Proline</td>
<td>0.24-0.48</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.21-0.24</td>
<td>Isoleucine</td>
<td>0.53-0.73</td>
<td>Serine</td>
<td>0.26-0.30</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>0.24-0.29</td>
<td>Glycine</td>
<td>0.25-0.26</td>
<td>Threonine</td>
<td>0.30-0.36</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.16-0.32</td>
<td>Lysine</td>
<td>0.12-0.24</td>
<td>Tryptophan</td>
<td>0.57-0.61</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.37-0.41</td>
<td>Leucine</td>
<td>0.61-0.71</td>
<td>Tyrosine</td>
<td>0.44-0.55</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.25-0.29</td>
<td>Methionine</td>
<td>0.51-0.60</td>
<td>Valine</td>
<td>0.44-0.66</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>0.31-0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

http://www.reachdevices.com/TLC_aminoacids.html

http://www.wpi.edu/Academics/Depts/Chemistry/Courses/General/tlc1.gif
Analysis Questions - on a separate sheet of paper complete the following

1. From reading your TLC plate, did either of the amino acid solutions contain phenylalanine? Which one?
2. What would you advise the patient and physician in terms of the possibility of the symptoms being caused by phenylalanine?
3. Why was it necessary to test the phenylalanine solution at the same time as amino acid solutions A and B?
4. Did the phenylalanine solution Rf value that was calculated match the Rf value in Data Table 2? If not, what may have caused the difference?
5. Why does touching the TLC plate with your hands potentially contaminate the results?
6. You dropped and mixed up your samples. You know that one container has valine, alanine, and glycine. The other container contains leucine, lysine, and methionine. How could you use TLC to determine which container has which amino acids?
7. You run a TLC plate with amino acid samples and obtain three Rf values: 0.70, 0.36, and 0.14. What are the possible amino acids in the sample?
8. CONCLUSION: In 1-2 paragraphs summarize the procedure and results of this lab.

Review Questions - on a separate sheet of paper complete the following

1. What are the four major types of macromolecules?
2. List the monomer for each of the four macromolecules.
3. How many essential amino acids make up proteins?
4. How is the structure of a protein similar to the structure of a paragraph?
5. What are the main functions of proteins in the body?
6. What is chromatography?
7. What is thin-layer chromatography?
8. How is the Rf value calculated?
9. What is the cause of phenylketonuria (PKU)? What are the symptoms of PKU?
10. In what food sources is phenylalanine found? Why is a warning label on food products with phenylalanine important?
11. What is the Rf value of tryptophan?
12. Look at Figure B. If an individual was not receiving enough glycine what organs could be affected?
13. What if they were not receiving enough methionine?