Experiment 8 CHEM 209 Lab, Spring 2005

AMINO ACIDS AND PROTEINS

Adapted from S. L. Saeger and M. R. Slabaugh, *Safety-scale Laboratory Experiments for General, Organic, and Biochemistry for Today*, 4th Ed., p. 387.

Materials Needed

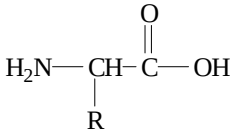
0.12% solutions of aspartic acid, phenylalanine, serine, and aspartame
Diet Coca-Cola, aged and fresh
1 mL 3M HCl
0.2% ninhydrin spray
5 mL 12:3:5 butanol:acetic acid:water solution
Ruler
one 9 x 6 cm TLC plate
gloves
capillary tubes
heat gun
drying oven
TLC Tank
100 mL fat-free milk
5 mL glacial acetic acid

Additional Reading

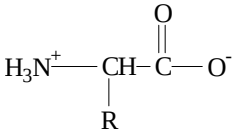
Denniston, chapter 19.

Introduction

Alpha amino acids are the building blocks of proteins. These compounds are all similar in structure because each has two characteristic functional groups: the carboxylic acid group (-COOH) and the amino group ($-NH_2$). They also contain a characteristic side chain that differentiates each amino acid from others. The general structure for an amino acid is shown. The R represents the side chain of the amino acid.



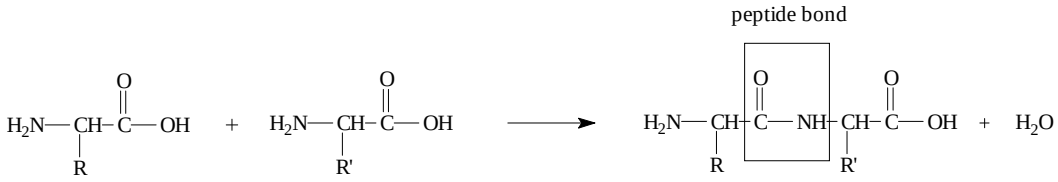
The structure shown above is simplified because amino acids generally do not exist as shown. Because both an acidic and a basic functional group are present, amino acids generally exist in *zwitterionic* form. That is, the acidic proton of the carboxylic acid is transferred to the nitrogen on the amino group, resulting in an ionic form that is soluble under aqueous conditions.



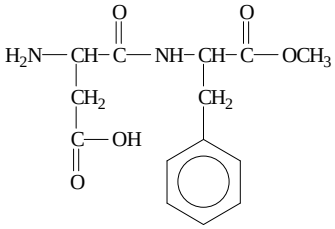
ZWITTERION

In fact, different pH conditions can lead to different forms of the acid. Under acidic conditions, both the carboxyl group and the amino group will be protonated. Under basic conditions, the carboxylate will be formed and the amino group will remain neutral. The ability for an amino acid to form several different types of ions explains its versatility in many cellular functions. In addition, the various R groups can contribute to its affinity for different solvents. Some R groups contain carbon and hydrogen atoms only which makes the amino acids nonpolar and hydrophobic. Others contain OH or SH groups that makes the amino acids hydrophilic. We can use the properties of the R groups to characterize the amino acids. The various solubilities provided by the side chains can be utilized to identify the different amino acids.

Peptide linkages hold amino acids together. Peptide linkages occur through *condensation* of two separate amino acid groups. The formation of each peptide bond requires the loss of one molecule of H_2O. Likewise, peptide bonds can be broken through hydrolysis to form individual amino acids.



In this experiment, we will determine the percentage of the protein, **casein,** in non-fat milk. This will be done by precipitating the protein with acetic acid and weighing the amount of protein obtained. We will also use *thin-layer chromatography* to identify **aspartame**, an artificial sweetener found in diet pop, and its hydrolysis products.

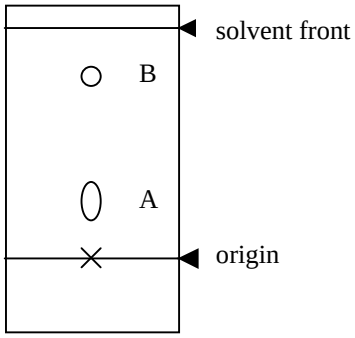


ASPARTAME

If you look closely at the structure of aspartame, you will notice that the terminal acid group is not a carboxylic acid, but rather the methyl ester of carboxylic acid. This type of variation in protein structure is very common throughout all types of amino acids. Therefore, upon hydrolysis, aspartame will yield aspartic acid, phenylalanine, and methanol.

Thin-layer chromatography is a useful analytical technique that allows various components of a mixture to be separated based on their polarity. Generally, a thin sheet of plastic or glass is used that has been coated with silica gel. Silica gel is a polar substance that will attract other polar substances. As a result, nonpolar substances will not have a strong affinity for the gel. A small drop of the mixture to be separated is applied near one end of the plat. The spotted end of the plate is then dipped into a developing solvent, called the *mobile phase*¸ which flows up the plate by capillary action. As the developing solvent flows up the plate, it can carry along the components of the mixture. The more soluble the component is in the solvent, the faster it travels up the plate. If it is not very soluble in the solvent, it will remain adsorbed on the surface of the silica gel, or *stationary phase*, and travel at a much slower rate.

The rates of flow are measure in terms of R_f values. An R_f is the ***relative*** distance that a sample component has moved relative to the distance moved by the developing solvent. The following illustration will demonstrate how the R_f is calculated. R_f is measured by dividing the distance the component traveled by the distance the solvent traveled. Therefore, an R_f value can never be greater than 1. You may want to review experiment #3 since we also used TLC in that lab.



$$R_f\,(A) = \frac{\text{distance A traveled}}{\text{distance solvent traveled}}$$

The R_f value for a particular component is characteristic for that component in that particular solvent. Therefore, it will always be the same and it can be identified in other mixtures. In this experiment you will determine the R_f values of three pure substances and compare these data with the results observed from diet pop samples in order to determine which of the substances are present in the diet pop.

PROCEDURE

Part I - Diet Coke analysis

1. Prepare a boiling water bath in a 250 mL beaker.
2. Obtain a TLC plate. Handle it carefully by the edges and do not bend. Using a lead pencil (not a pen), ***lightly*** draw a line across the plate about 1 cm from the bottom. ***Lightly*** mark 5 positions where your samples will be spotted. You may want to number the spots to number the spots to keep them straight.
3. Pour 5 mL of the butanol:acetic acid: water solvent mixture into a TLC tank. Place a piece of filter paper in the mixture to get it wet and prop the filter paper up along the side. Cover the tank with the provided plastic cap.
4. Reference solutions of each of the following substances will be provided: phenylalanine, aspartame, and aspartic acid. Two Diet Coke samples, a fresh one and an aged one will also be provided. You will use these samples for spotting the plate.
5. Make spots of phenylalanine, aspartic acid, aspartame, fresh Diet Coke, and aged Diet Coke at the positions marked on the plate in step 2. Try to let the spots only spread out to about 1 mm in diameter. Be sure to keep a log of which spot contains which sample! Multiple spotting will be required for the Diet Coca-Cola samples (about 12-15 times.) Allow the spot to dry each time before reapplying in order to prevent the spotting area from getting too large. Make sure you keep track of which spot is which and allow the spots to dry.
6. Place the spotted TLC plate in the developing tank you prepared in step 3. Make sure that the spots applied to the plate are above the surface of the eluting solvent. The developing process will take a long time. During this time, continue on with the isolation of casein. Watch the plate carefully. When the solvent is about 1 cm from the top, it can be removed from the chamber.
7. Allow the solvent to evaporate off of the plate (in the fume hood!) The drying process can be accelerated by using an air gun.
8. Using gloves, spray the plates with ninhydrin solution holding the solution about 6 inches away from the plate. Place the sprayed plates in a drying oven for 2-3 min.
9. Remove the plates from the oven, measure the distances moved of all spots and the solvent front.

Part II - Isolation of casein

1. Place 100 mL (100 g) of fat-free milk in a 125-mL Erlenmeyer flask. Weigh the flask before and after so that you can get a precise mass.
2. Heat the milk to 50°C in a hot tap water bath.
3. Add glacial acetic acid dropwise to the warm milk. The acid will cause the protein to precipitate out of solution, forming a clump and a clear solution. Add acid until no further precipitation occurs. **Caution:** Glacial acetic acid is extremely corrosive! Wear gloves and be careful not to spill any. If you get some on your skin/clothes, rinse off immediately with plenty of water.
4. Allow the sample to cool. Then decant the liquid down the drain, flushing with water from the faucet. Dry the solid with paper towels. Allow the protein to dry for a few minutes.
5. Weigh and record the mass of casein from the milk sample.

PRE-LABORATORY QUESTIONS

Experiment 8
AMINO ACIDS AND PROTEINS

Names _____Section_____ Date _____

1. Give the structures of phenylalanine and aspartic acid.

2. Measure and calculate the R_f values of the two spots (A and B) shown in the hypothetical TLC plate shown on page 2 of the introduction. Show the calculations below.

OBSERVATIONS AND DATA

Experiment 8
AMINO ACIDS AND PROTEINS

Names _____Section_____ Date _____

TLC Results

Distances moved:

solvent _____

Phenylalanine _____

Aspartic acid _____

Aspartame _____

Fresh Diet Coke _____

_____

_____

Aged Diet Coke _____

_____

_____

Sketch of developed TLC plate

The sketch should be as realistic as possible. It should be done to scale and not only show the positions of the spot but their sizes and relative darkness as well.

TLC Observations:

Isolation of Casein

Mass of milk used _____ Mass of Casein obtained _____

Observations:

REPORT SHEET

EXPERIMENT 8 - AMINO ACIDS AND PROTEINS

Names _____Section_____ Date _____

Results Table for Part I – TLC of Diet Coke samples – R_f values Obtained

	spot 1	spot 2	spot 3
Phenylalanine			
Aspartic acid			
Aspartame			
Fresh Diet Coca-Cola			
Aged Diet Coca-Cola			

Show R_f calculations here

Results Table for Part II – Isolation of Casein

	experimental % of casein in nonfat milk	literature % of casein in nonfat milk	appearance of casein product
casein product			

Show the calculation of the percentage of casein in nonfat milk here. (% casein = $\frac{\text{mass of casein}}{100 \text{ g milk}} \times 100$)

Questions (Write neatly or type on a separate sheet of paper.)

1. Which of the three reference compounds (phenylalanine, aspartic acid, aspartame) were found in the fresh Diet Coke? Which were found in the aged Diet Coke. Use R_f values to support your conclusions. Are these results what should have been expected? Explain.
2. The shelf life of Diet Coca-Cola is ~3 months. Explain using a chemical equation.
3. Why does the milk protein (casein) precipitate when acetic acid is added?
4. Compare the experimental value you obtained for the percentage of casein in milk to a literature value and discuss.