Chem 210, Winona State University, Fall 2022

EXPERIMENT 10

PROTEINS – Isolation and Testing of Casein

Adapted from https://www.chemistry.mcmaster.ca/~chem2o6/labmanual/expt11/2o6exp11.html

Materials Needed

Powdered non-fat dry milk (5 g)
10% acetic acid(aq) (≈2 mL)

diethyl ether (5 mL)	Ethanol (5 mL)	1M NaOH(aq) (0.5 mL)
100 mL beaker	magnetic stirbar	stirrer/hotplate
0.1% Ninhydrin solution	2% egg albumin (1 mL)	0.1 M glycine (1 mL)

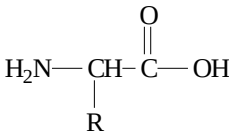
Buchner funnel, filter paper, vacuum tube, vacuum adapter, 75-mm test tubes (4)

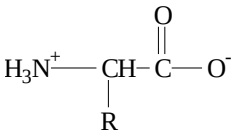
Additional Reading

1. Smith, chapter 21.
2. https://www.researchgate.net/figure/Typical-amino-acid-composition-of-whey-casein-and-soy-isolates_tbl2_227249571

Introduction

Alpha amino acids are the building blocks of proteins. These compounds all have the structure shown below left with both a carboxylic acid group (-COOH) and an amino group ($-NH_2$) linked together by a single carbon atom (the alpha carbon). There are 20 commonly occurring amino acids in biological organisms. These differ only with respect to the R group attached to the alpha carbon. The R groups or "side chains" can be simple non-polar alkyl groups (methyl, sec-butyl, isobutyl) or more complex groups with additional polar functional groups present (-SH, -COOH, $-NH_2$). Ironically, the side chain R group can also be a simple hydrogen atom.

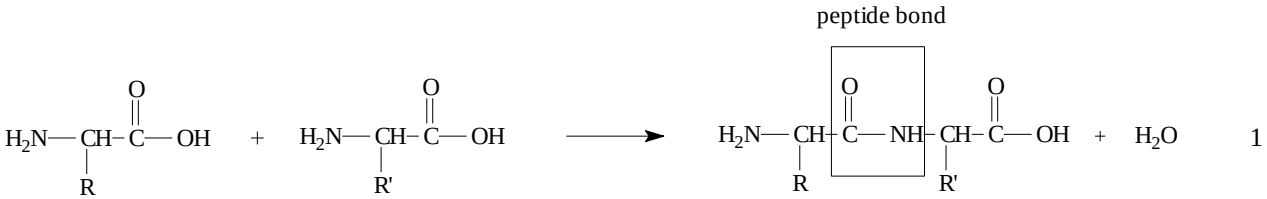




ZWITTERION

The structure shown above at left is oversimplified and unrealistic. Because both an acidic and a basic functional group are present, amino acids generally exist as *zwitterions*. 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 in water.

Amide linkages hold amino acids together in chains known as polypeptides or proteins. These amide linkages (also referred to as peptide linkages) come about through the *condensation* of an amino group on one amino acid with the carboxylic acid group on another. The formation of each peptide bond requires the loss of one molecule of H_2O (see equation below). Likewise, peptide bonds can be broken through hydrolysis to form individual amino acids (reverse reaction of equation below).



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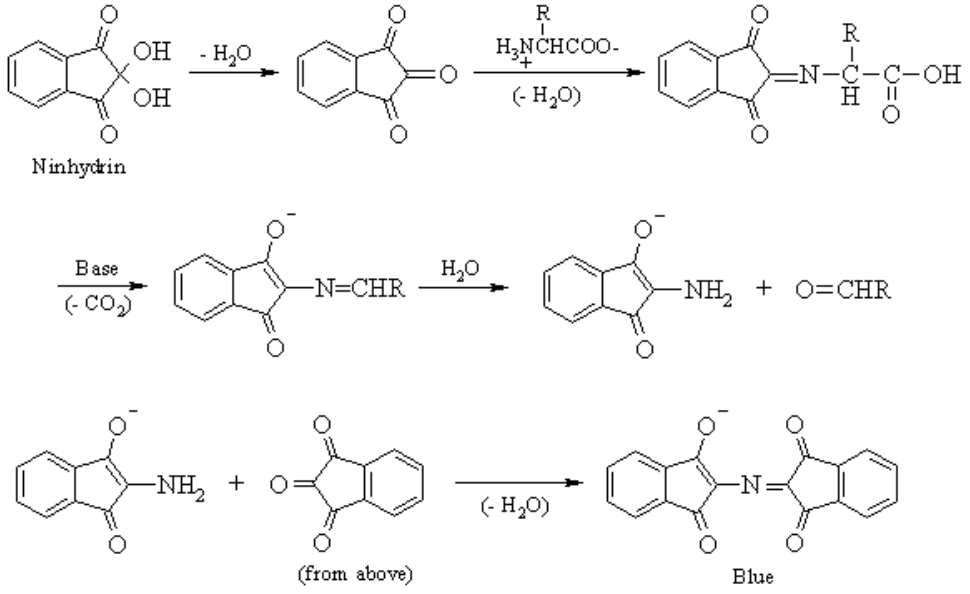
Milk is the most nutritionally complete food found in nature. All kinds of milk contain vitamins, minerals, proteins (mostly casein), carbohydrates (principally lactose), and lipids (fats). The amounts of these nutrients present in different types of milk differ greatly, however. Human milk contains less than half of the proteins and minerals of cows' or goats' milk for example.

There are three kinds of proteins in milk: *caseins*, *lactalbumins*, and *lactoglobulins*. All three are "complete proteins", so-called because they contain all the amino acid needed to sustain life and provide normal growth. These proteins not only contain more amino acids than plant proteins, but they contain greater amounts of amino acids than the proteins in eggs and meats.

Casein, the main protein in milk (approx. 80% of total protein mass), is a phosphoprotein, meaning that phosphate groups are attached to the hydroxyl groups of some of the amino acid side-chains. Casein exists in milk as the calcium salt, **calcium caseinate**. It is actually a mixture of at least three similar proteins which differ primarily in molecular weight and the amount of phosphorus groups they contain. The different caseins are known to aggregate together to form **micelles** in which the phosphate groups and associated calcium ions are on the outside and amino acids with non-polar side chains (valine, leucine, isoleucine) are on the inside.

Due to the presence of a large amount of acidic side chain R groups (glutamic acid is the most abundant amino acid at 20%) casein has an isoelectric point of pH 4.6. The pH of milk is about 6.6; therefore, casein has a negative charge at this pH and is solubilized as a salt. If acid is added to milk, the negative charges on the outer surface of the casein micelles are neutralized (by protonation of the phosphate groups) and the neutral protein precipitates, with the calcium ions remaining in solution:

The ninhydrin test is used to detect the presence of amino acids and proteins containing free amino groups. When heated with ninhydrin, these molecules give characteristic deep blue colors (or occasionally pale yellow). The reactions involved in this test are shown below.



In this experiment, we will isolate the protein, **casein,** from skim milk. This will be done by precipitating the protein with acetic acid and collecting the solid protein on a Buchner filter. The percent casein in skim milk will be determined and the casein will be tested using the Ninhydrin test.

PROCEDURE

Isolation of casein

1. Weigh out 5 g of non-fat dry milk and suspend it in 20 mL distilled water in a 100 mL beaker.
2. Add a magnetic stirbar and place the beaker on a hot plate.
3. Raise the temperature to 55°C (do not exceed).
4. Add 10% acetic acid drop by drop with stirring. Continue the addition until the liquid changes from milky to almost clear and the casein no longer separates. (Slightly less than 2 mL will be required),
5. Stir the precipitated casein until it forms a large amorphous clump; then remove it with tongs and place it in another beaker.
6. Set up a vacuum filtration apparatus and transfer the casein into the Buchner funnel so as to remove as much water as possible. Further dry it by pressing down on the solid with a spatula or the bottom of a small flask.
7. Place the dry casein in a 100 mL beaker and add 5 mL of a mixture of 1:1 ethyl ether and ethanol. Stir for 2-3 min then decant the ether into a waste beaker. Repeat the process with a second 5 mL portion 1:1 ether/ethanol. (These washes remove any small quantities of fat that may have precipitated with the casein.)
8. Again, suction filter the product. Place the casein between several layers of paper towels to help dry the product, and let it air dry for 10-15 min.
9. Weigh the casein and calculate your percent recovery.

Ninhydrin test

1. Place approx. 100 mg of your casein in a 50-mL Erlenmeyer flask with 7 mL of water and 0.1 mL of 1M NaOH, stopper the mixture, shake it to ensure solution of as much of the casein as possible,
2. Into separate, labelled 75-mm test tubes place 1 mL of each of the following; your just prepared casein solution, 2% egg albumin, 0.1 M glycine, distilled water.
3. Add 4 drops of 0.1% ninhydrin solution to each tube. (**CAUTION: NINHYDRIN IS TOXIC – WEAR GLOVES**)
4. Add a boiling chip to each test tube and heat to 100°C on a hot-water bath. Record your observations.

PROTEINS – Isolation and Testing of Casein

PRE-LABORATORY QUESTIONS

Name _____Section_____ Date _____

Draw the structures of glutamic acid, valine, leucine, and isoleucine all in zwitterion form as they exist at pH 7.

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IN-LAB OBSERVATIONS/DATA

Names _____Section_____ Date _____

Isolation of Casein

Observations

Data

Mass of non-fat dry milk used _____

Temperature solution (steps 3-4) _____

Time required for acetic acid addition _____

Mass of Casein obtained _____

Percent recovery _____

PROTEINS – Isolation and Testing of Casein

REPORT SHEET

Names _____Section_____ Date _____

Results Table

Mass (g)		Casein product		% of casein in nonfat milk		
Powdered milk	Casein product	Appearance	Ninhydrin test	exptl	lit	% error
					12%	

Show the calculation of the percentage of casein in nonfat milk here.

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

1. Why does the casein precipitate when acetic acid is added?

2. Compare the experimental value you obtained for the percentage of casein in powdered non-fat milk to the literature value and discuss. How accurate was the experiment in terms of determining this value?

3. Discuss the results of the Ninhydrin tests and what they mean.