

Biology 3A Laboratory Protein Fingerprinting using SDS-PAGE

Objectives:

- To use polyacrylamide gel electrophoresis (PAGE) to compare protein profiles derived from muscle tissue of different seafood items.
- To understand how DNA sequence changes can occur and pass from generation to generation.
- To relate DNA sequence changes to protein composition and organismal form change over time.
- To construct an evolutionary tree based upon morphological characteristics.
- To determine which proteins are in each sample and their molecular weights.



Allergy Warning: Students with known shellfish allergies should avoid all potential contact with shellfish samples.

Introduction

Charles Darwin proposed the revolutionary idea that varieties of forms and species are derived from a common ancestor – descent with modification. When a population of similar, related individuals acquires new characteristics over many generations, we refer to that process of modification over time as evolution. The millions of species living on earth today have one amazing, strikingly commonality: we all use essentially the same chemical language and mechanisms for metabolism and reproduction. Reliable fossil evidence for ancient bacteria, the earliest known cellular life, dates to at least 3.5 billion years ago. Since then, the expansion and modification of the gene pool of the original ancestral organisms, has resulted in the many millions of different species that exist on the planet today. Since DNA contains the information to make proteins, mutations or alterations in DNA can produce new proteins that function differently. These changes can lead to novel traits and diversity among related organisms. This genetic diversity makes evolution possible, since natural selection favors some individuals and not others, and so leads to changes in the composition of the gene pool over time (evolution). DNA both provides for the continuity of traits from one generation to the next and accounts for the variation that can lead to differences within a species and even to entirely new species (speciation).

Extensive studies have revealed that a great deal of DNA sequence similarity exists among the genes of all modern day organisms. For example, scientists were astounded to discover that the same family of genes (Hox genes) controls the embryonic development of animals as diverse as fruit flies, zebra fish and humans. The highest level of genes sequence similarities among diverse organisms can only be due to common ancestry or homology. When the proteins of many diverse organisms are compared, they too show similarities, indicating that they have evolved by the shuffling and modification of simpler protein domains. Families of related genes and proteins are found distributed wide and far – deep in the sea, underneath rocks, and flying above us in the sky. So the question isn't whether evolution happens, but rather how it happens?

Most of you are familiar with the traditional classifications (domain, kingdom, phylum class, etc.) of organisms which have been based primarily on morphological characteristics – traits

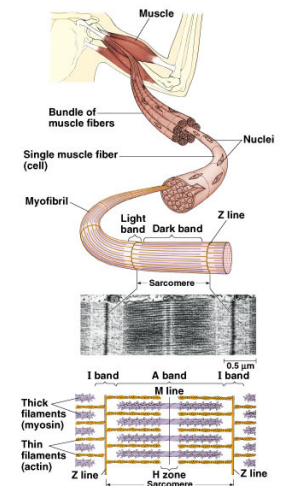
that can be seen with the unaided eye or microscope. Darwin's ideas about evolution were inspired by his observations of the large variety of beaks among finches. In 1859, he did not even know about DNA because no one really did either. However, he did have some insight. A century later, scientists discovered the central framework of molecular biology: DNA (genotype) → RNA → proteins → trait (phenotype), and came to understand the cause of these all-important differences.

Molecular Framework of Biology: DNA → RNA → Protein → Trait

DNA and RNA have informational roles in cells, whereas proteins and traits are the production of the information. As you may recall, during transcription, DNA sequences encoding individual proteins are copied to produce mRNA. Then during translation, mRNA sequences are read by cellular machinery that synthesizes amino acid chains of precise order and content, as specified by the mRNA. These amino acid chains may be further modified within the golgi complex to become the proteins that form structures and carry out the biochemical functions that we observe as traits.

Proteins have a wide variety of functions from enzymes that assist in biochemical reactions, to structural support and even locomotion (refer to your lecture material for other functions). In this lab we will examine protein in muscle tissue. Muscle tissue consists of many different proteins with specialized functions, but the contraction of muscle results primarily from the interaction of two predominant proteins, actin and myosin. Actin and myosin form muscle fibers, the biochemical machinery that causes muscles to contract. These two proteins make up the structure and function of muscle that is common to all animals (Figure 1).

Figure 1: Expanded view of muscle structure. Thick myosin filaments and thin actin filaments form myofibrils, which are bundled together to form muscle fibers.



The genes for actin and myosin are members of gene families that encode proteins that enable movement. Other proteins associated with muscle have known, unknown, or speculated functions and may vary in their occurrence among different species. The variations in an organism's proteins are the results of random DNA mutations within the encoding genes (exons) which have occurred over thousands to millions of years. Each mutation may result in some kind of change in a protein. The changes can be beneficial, neutral or detrimental to an organism's ability to survive and reproduce.

How will you see a protein molecule?

In this investigation, you will use high-resolution polyacrylamide gel electrophoresis (PAGE) to display the various proteins in the muscle tissue of different seafood items and to perform molecular weight determinations. By the displays of the proteins assortments in the different seafood

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items, you can test the hypothesis that proteins are indicators of genetic and therefore, evolutionary relatedness.

You will use a combination of a detergent and heat to extract and denature (disrupt) the proteins in several fish muscle samples. The ionic detergent, sodium dodecyl sulfate (SDS), coats dissolved proteins and polypeptides with negative charges. The SDS-coated proteins then all move toward the positive electrode (anode) but at different rates depending on their sizes.

When the functional proteins are coated with SDS and heated, they lose their three-dimensional structure and take on a net negative charge. Bigger polypeptides are coated with more molecules of SDS so the ratio of a protein's molecular weight to its charge is approximately the same for all proteins. This means that size becomes the determinant of mobility through the gel.

Many large proteins are made of smaller protein subunits. These polypeptides are held together by bonds between sulfur atoms in the amino acids. Certain chemical treatments break these disulfide bridges and release the separate polypeptide units. Thus, one functional, native protein can give rise to several smaller polypeptides. Myosin, for example, is a complex of 2 heavy protein chains and 4 light chains. The light chains are of two different sizes, so a purified myosin sample will form 3 separate bands when treated appropriately prior to electrophoresis.

Determining the size of proteins via electrophoresis

To analyze the protein the fingerprint, we will use polyacrylamide instead of agarose gels. The gel matrix formed by polyacrylamide is much tighter and able to resolve much smaller molecules than agarose gels. Polyacrylamide gels have pore sizes similar to the size of proteins. DNA molecules are orders of magnitude larger than proteins and for nucleic acids, agarose is usually the preferred gel medium. However, when separating very small fragments of DNA, for example during DNA sequencing, polyacrylamide is the gel matrix of choice.

Untreated or native proteins will migrate in a gel at rates based on both their electrical charges and their masses. If we equalize charge-to-mass ratios (charge densities) of all protein molecules, mass becomes the only factor determining the migration rate of each protein. This is accomplished by treating the proteins with the ionic detergent SDS, which is present in both the gel running buffer and the sample loading buffer. This technique is called SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Protein size is measured in daltons, a measure of molecular weight. One Dalton is defined as the mass of a hydrogen atom, which is 1.66×10^{-24} gram. Most proteins have masses on the order of thousands of daltons, so the term kilodalton (kD) is often used to describe protein molecular weight. Given that the average weight of an amino acid is 100 daltons, the number of amino acids in a protein can be approximated from its molecular weight.

What can proteins tell you about evolution?

The banding patterns on your gel reveal information about the protein composition of muscle from your seafood samples. Proteins are synthesized according to the genes of an

organism's DNA. Since closely related organisms share similar DNA sequences, the proteins encoded by their DNA should be very similar as well, and similar protein compositions should be reflected in banding patterns.

Different branches of related organisms separated at different evolutionary times. The further apart species are on the tree, the less related they are. Mollusks and arthropods diverged from one another before the emergence of chordates, animals with backbones, very early in evolutionary time. These animals are only distantly related to fish, birds, reptiles, mammals and amphibians, which are more closely related to each other (Figure 2).

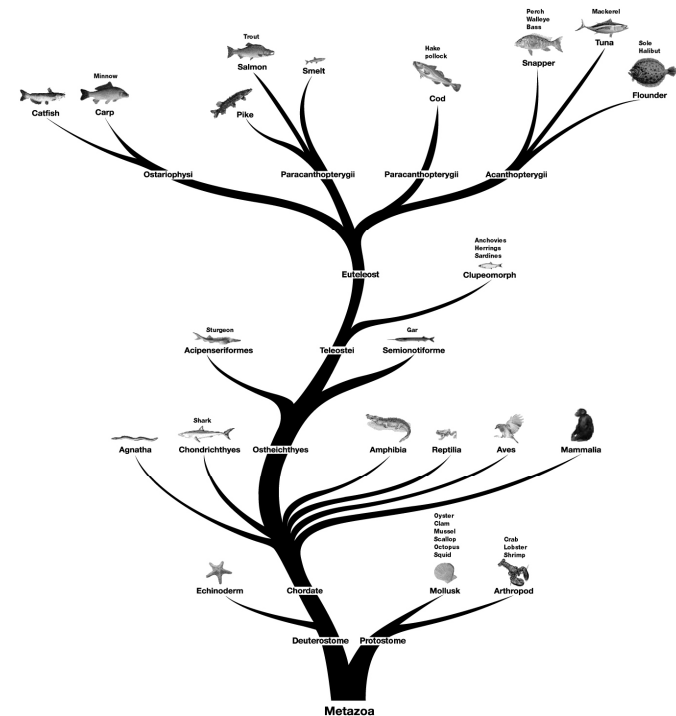


Figure 2: fish evolutionary tree based on Ichthyology Web Resources and the Tree of Life.

Procedures

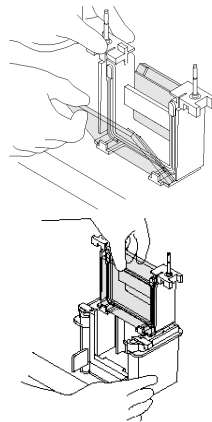
Extraction & Denature Proteins:

1. Use an indelible pen to label one 1.5 mL **flip-top** microtube for each of the seafood samples. Also label one **screw cap** micro tube for each sample.
2. Add 250 μ L of Laemmli sample buffer to each labeled **flip-top** microtube.
3. Cut a piece of fish muscle 0.25 x 0.25 x 0.25 cm³ or about the size of a "Tic-Tac" (☐) and transfer each piece into a labeled **flip-top** microtube. Close the lids. Avoid taking any skin, fat or bones.
4. Flick the microtubes 15 times to agitate the tissue in the sample buffer.
5. Incubate the microtubes for 5 minutes at room temperature to extract and solubilize the proteins.
6. Carefully transfer the Laemmli sample buffer by pouring from each **flip-top** microtube into a labeled **screw cap** microtube. Do not transfer the chunk of fish! It is not necessary to transfer all of the fluid to the screw cap tube. Only a small volume will be needed to load and run the gel.
7. Obtain the Kaleidoscope prestained standard (KS) and the actin and myosin standard (AM) from your instructor.
8. Heat the fish samples and the actin and myosin standard (AM) in screw cap microtubes for 5 minutes at 95°C in a water bath.
9. You may store all the samples at room temperature if they are to be loaded within 3 – 4 hours or store them at -20°C for several weeks.

Electrophoresis:

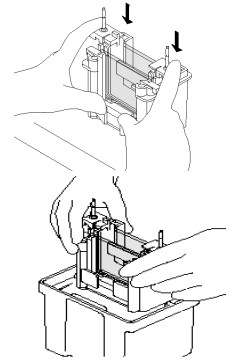
10. Set up the electrophoresis module:

- a. Prepare a Ready Gel cassette by cutting along the black line on the bottom of the cassette with a razor blade and pulling off the plastic strip, as indicated on the gel cassette.
- b. Remove the comb from the Ready Gel cassette.
- c. Place the cassette into the electrode assembly with the plate facing inward. Place a buffer dam or another gel cassette on the opposite side of the electrode assembly, with the notch on the buffer dam facing inward.



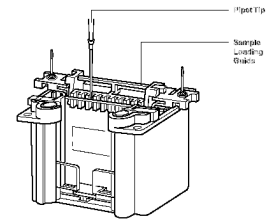
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- d. Slide the gel cassette, buffer dam, and electrode assembly into the clamping frame.
- e. Press down on the electrode assembly while closing the two cam levers of the clamping frame.
- f. Lower the inner chamber into the mini tank.
- g. Completely fill the inner chamber with 1x TGS electrophoresis buffer, making sure the buffer covers the short plate. It takes about 150 mL of buffer.
- h. Pour approximately 200 mL of 1x TGS electrophoresis buffer into the mini tank.
- i. Place the yellow sample loading guide on top of the electrode assembly.



11. Load samples in your gel and record the names of your fish samples.

Lane	Volume (μ L)	Sample
1	10	Fish sample 1
2	10	Fish sample 2
3	10	Fish sample 3
4	10	Fish sample 4
5	10	Kaleidoscope standard (KS)
6	10	Actin & myosin standard AM)
7	10	Fish sample 5
8	10	Fish sample 6
9	10	Fish sample 7
10	10	Fish sample 8



12. Electrophoresis at 30 minutes at 200 V. (Your instructor will give you directions.)
13. After electrophoresis, remove the gel from the cassette and transfer the gel to a container with a large volume of DI water and agitate gently for 5 minutes. Rinse 3 – 4 times. Use gloves to avoid transferring human proteins to the gel.
14. Add 40 mL Bio-Safe Coomassie Blue stain and stain the gel for 1/2 - 1 hour, with gentle shaking for best results.
15. Pour off the blue stain. To destain the gel, add enough water to the dish containing the gel to fill the dish to about the half way point. Change the water in the dish several times if possible. Allow the gel to destain overnight, with gentle shaking for best results. After destaining, blue bands should be distinct on a clear background.
16. Dry or photograph your gel following your instructor's directions.

This laboratory guide was adapted from;
Biotechnology Explorer Protein Fingerprinting Instruction Manual,
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