3. Myosin is a very large protein molecule (500 kdal) that makes up the thick filaments of muscle tissue. It contains two identical heavy chains (200 kdal each) and four light chains (about 20 kdal each). During sample preparation, and with the use of a reducing agent, it breaks down to its heavy- and light-chain fractions.

   a. Using the standard curve plot, locate the heavy- and light-chain bands of the walleye samples on the gel. How far should the heavy- and light-chain bands have migrated?

   b. How many bands do you expect to find for the heavy and light chains? Explain your answer.

   c. The average amino acid residue in a protein has a molecular weight of 120 Daltons. How many amino acid residues make up the heavy chains? the light chains?

4. What can be inferred from the protein banding pattern on the gel?

4. On a sheet of semilog graph paper, draw a standard curve plot using the molecular weight protein marker electrophoretic data. Plot the R_f (X axis) against the molecular weight of each fragment (Y axis).
5. From the standard curve, determine the molecular weight of the unknown polypeptides.

Note: *The gel is 70mm long. Therefore, R_f values are calculated as migration distances in millimeters divided by 70mm.*

High Molecular Weight Protein Markers

Fragment	Molecular Weight (Daltons)	Distance Migrated (mm)	R_f
1 (Urease)	120,00		
2 (Fructose-6-P-Kinase)	83,000		
3 (Catalase, Bovine Liver)	60,000		
4 (Bovine Serum Albumin)	66,000		
5 (Egg Albumin)	45,000		
6 (Carbonic Anhydrase)	29,000		

Questions

1. How is genetic distance of the three fish samples determined through comparison of the banding pattern of the treated samples with the banding pattern of the untreated samples?

2. How are the electrophoretic patterns of the three samples similar? How are they different? Which two species have a similar banding pattern? What does this indicate? Explain your answer.

Analysis

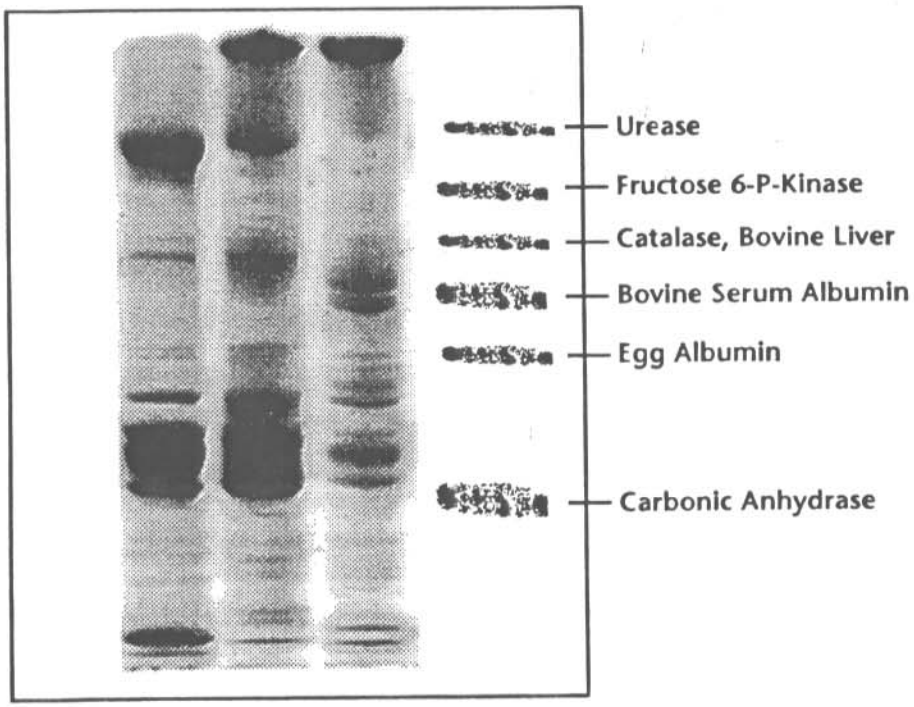
In protein separation by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, migration is determined not by intrinsic electrical charge of polypeptides, but by molecular weight. SDS is an anionic detergent that denatures proteins and confers a negative charge on the polypeptide in proportion to its length. Treating proteins with SDS and a reducing agent causes the individual polypeptides to become negatively charged with equal charge per unit length. It is possible to determine the molecular weights of polypeptides by running a gel with standard proteins of known molecular weight along with unknown polypeptides. A linear relationship exists if the logarithms of the molecular weights of the standard proteins are plotted against their respective relative mobilities (R_f). To determine the R_f of a protein, divide its migration distance form the top of the gel to the center of the protein band by the migration distance of the tracking dye from the top of the gel.

$$R_f = \frac{\text{Distance protein has migrated}}{\text{Distance tracking dye has migrated}}$$

A standard curve is constructed by plotting the R_f values of the standard polypeptides against their molecular weight. The R_f values of the unknown polypeptides is determined in the same way. The molecular weight is read directly from the standard curve.

Below is a representation of an electrophoresed gel imaging fragments of a protein marker.

Figure 2
Electrophoresed Gel



1. Measure the distance, in millimeters, from the top of the gel to each fragment. Measure from the top of the gel to the middle of each fragment band. Record each, in the table below, in order, beginning with the band closest to the top of the gel.
2. Measure the distance from a sample well to the end of the gel.
3. Calculate the R_f of each fragment; record data in the table below.

Note: *The distances from individual wells to the end of the gel may not be the same. Therefore, the R_fs should be calculated based on well distances measured from individual lanes.*

5. Slowly fill the outer compartment with working running buffer concentrate until it reaches the same level as the inner compartment.
6. Load 20µl from each sample tube onto a corresponding gel lane with a micropipet. Do not pierce the bottom of the wells with the micropipet tip. Do not overload wells. Keep track of the well number, from left to right, where each sample is placed.

   Lane #1: Yellow Perch
   Lane #2: Walleye
   Lane #3: Chinook Salmon
   Lane #4: High Molecular Weight Protein Marker

   Note: *The amount of protein in the reaction tubes is extremely small. Your instructor will demonstrate the correct procedure needed to transfer samples from these reaction tubes to the wells on the gel.*
7. Making sure the cover is dry, attach the lid to the chamber, making sure it is aligned properly. Wipe off any spills on the apparatus before proceeding to the next step.
8. Making sure that the patch cords attached to the cover, as well as the female plugs and the banana jacks on the chamber, are completely dry, connect the red patch cord to the red electrode terminal on the power supply. Connect the black patch cord to the black electrode terminal on the power supply.

   Note: *Be sure your instructor checks the connections before proceeding to the next step.*
9. Plug in the power supply and set it to 125V.
10. Turn on the power supply. The red power light will illuminate, and bubbles will form along the platinum electrodes.
11. Observe the migration of the sample down the gel toward the red electrode. Turn off the power when the loading dye has neared the end of the gel. Unplug the power supply.
12. Wait approximately 10 seconds, then disconnect the patch cords first from the power supply and then from the chamber.
13. Remove the lid assembly and pull out the wedges, then the gel cassettes.
14. Using a scalpel, cut the yellow tape longitudinally along one side of the cassette and open the cassette.

    Note: *It may be necessary to insert the edge of the scalpel between the glass plates and gently pry them apart.*
15. Put a notch in the lower left corner of the gel to ensure the lanes can be identified after the gel is removed from the unit.
16. Lift the gel tray with the gel from the chamber and gently place the gel in the staining tray.
17. Pour enough stain into the tray so the stain just covers the gel; let gel stain for approximately 15 minutes.
18. When the gel has completed staining, carefully decant the used stain. Add distilled water to the staining tray. To accelerate destaining, gently rock the tray. Destain overnight or until bands are distinct, with little background color.
19. View the gel against a light background, such as white paper, or on a light table. Gels can be stored, refrigerated, in self-sealing plastic bags.

    Note: *Wash hands thoroughly before leaving the lab.*

Objective

To electrophorese samples of three common fish—perch, walleye, and salmon—and compare their electrophoretic banding patterns to determine their evolutionary relationships. Also to construct a standard plot curve using molecular weight protein markers as well as determine the molecular weight of the samples.

Preparation Notes

Safety: *The power supply produces a high enough voltage to cause severe electrical shock if handled improperly. For safe operation, follow all directions and precautions. Examine all components of the electrophoresis apparatus prior to each use: all cords, plugs, jacks, the electrophoresis chamber itself, and the power supply. Be sure you are well acquainted with the correct operation of the unit and know how to make proper electrical connections. Wear personal protective equipment: safety goggles and smocks or aprons while loading gels and during electrophoresis, and protective gloves when staining.*

Materials Needed per Lab Group

Samples in Labeled Microcentrifuge Tubes
- Tube #1: Yellow Perch
- Tube #2: Walleye
- Tube #3: Chinook Salmon
- Tube #4: High Molecular Weight Protein Marker

1 SDS Polyacrylamide Gel, 6–14%
1 Capillary Micropipet, 20µl
1 Pipet, 1ml
1 Gel Staining Tray
1 Metric Ruler
Calculators
Semilog Graph Paper
Goggles
Gloves
Aprons

Shared Materials

WARD'S Vertical Dual-PAGE Chamber
Power Supply
SDS Running Buffer Solution 1X, 1L per Chamber
WARD'S Protein Stain, 100ml per gel
Deionized or Distilled Water
1 Gel Handler Spatula

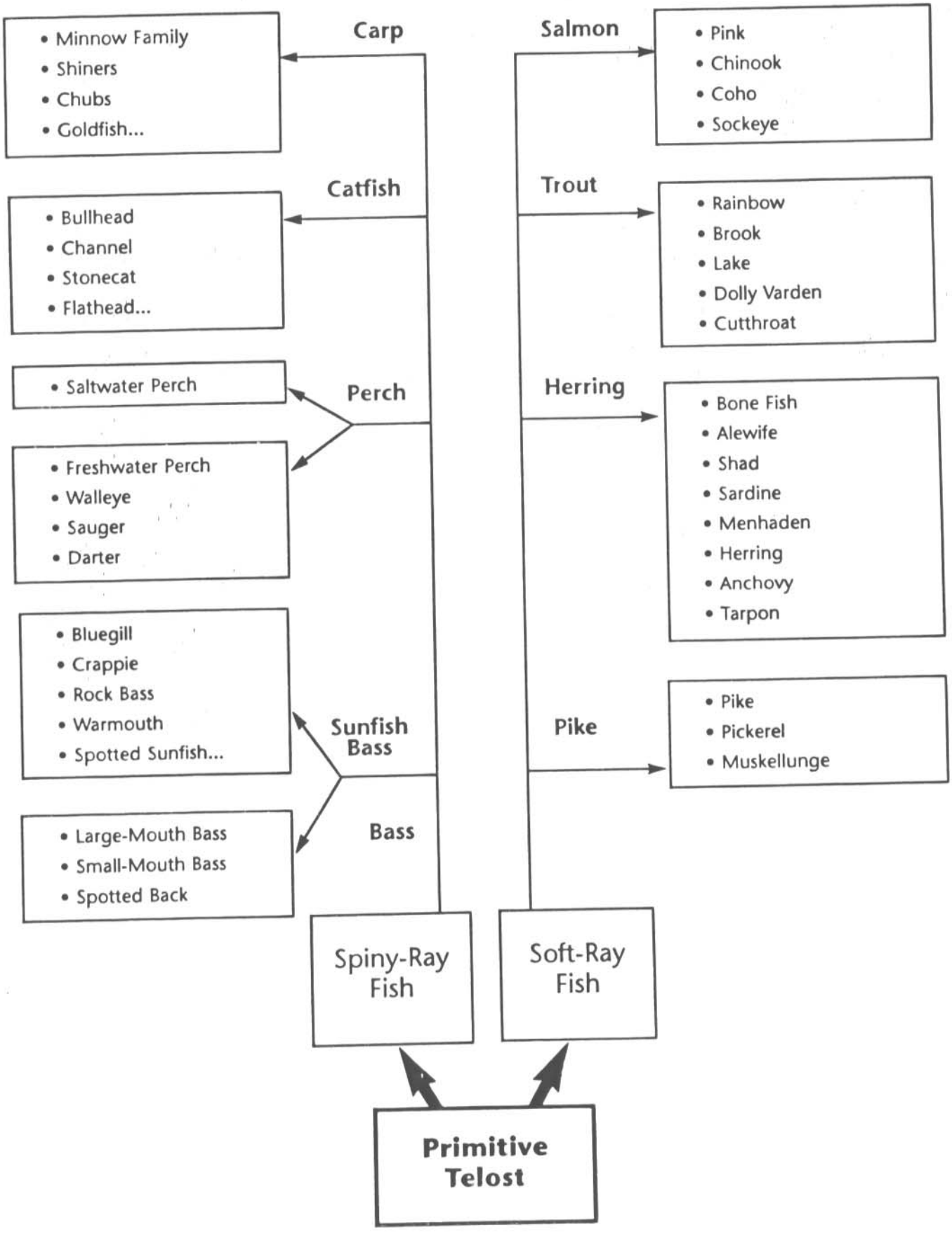
Procedure

1. Remove the gel from the package and remove the comb from the gel.
2. Place each gel into the outer chamber of the tank, making sure the wells are facing inward, toward the inner compartment.

   Note: *If you are running only one gel, insert the included plexiglas plate in the other outer chamber, in place of a second gel.*

3. Insert the wedges into each corner, making sure the gel is snug against the gasket to prevent leaks into the outer compartment.
4. Fill the inner compartment with working buffer solution until it covers the wells. Using a pipet, flush out any air bubbles in the wells.

Figure 1
Evolutionary Relationship of Some Common Fish



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Introduction

Genetic divergence during vertebrate evolution has been studied using a variety of methods, most recently gel electrophoresis. Individual polypeptides that differ by as little as a single amino acid can be resolved on a polyacrylamide gel based on their molecular weight. The banding pattern of proteins from one species of animal can be compared to another species to determine the genetic distance between the two species. Many of the bands imaged on the gel are composed of several polypeptides linked together by disulfide bridges to form more complex proteins. The production of these proteins involves the interaction of several genes to form a functional unit. These proteins resist evolutionary change and are more conserved in nature than individual polypeptides. In addition, muscle tissue is a functionally fixed structure that does not undergo much selection pressure through time. The banding pattern on a gel can show similarities that go farther back in the evolution of various species than can a gel that contains only individual polypeptides.

An example of genetic divergence is that of fish. More than 70 million years ago, the first bony fish (telosts) split into two major groups—the soft-rayed fish and the spiny-rayed fish. The soft-rayed fish developed into herrings, trout, salmon, and pikes. The spiny-rayed fish gave rise to perch, catfish, bass, and carp. For example, although they all developed from the same ancestor, salmon, perch, and walleye are all different. Salmon spend most of their adult lives in the ocean, returning to fresh water to spawn and die. They have a lifespan of four to seven years and can reach a weight of up to 70 pounds. Their physical characteristics include very small scales and a long posterior fin. Perch are small, shallow-water fish found in lakes, ponds, and slow-moving streams. They are easily recognized by the six to nine blackish bars on the sides of their bodies. When fully mature, they measure 4 to 12 inches in length and weigh only slightly more than one pound. Walleye have a mottled pattern with a black blotch at the posterior end. Although they belong to the perch family, they can achieve a length of 30 inches and weigh up to 25 pounds.

Electrophoresis results show that the banding patterns of yellow perch and walleye are similar, indicating that they derived from a common ancestor fairly recently—several million years ago—whereas the salmon banding pattern is different from those of the perch and walleye.