

Laboratory 6A

Hemoglobin Polymorphism and Mendelian Genetics**Objectives**

1. Describe in writing and in symbols the genetics of sickle cell anemia
2. Describe in writing and in drawings the structure of human hemoglobins
3. Describe the process of electrophoresis and its use as a diagnostic tool for detecting differences between proteins such as hemoglobins

Introduction

Hemoglobin is the protein in our red blood cells (RBCs) that is responsible for the transport of oxygen. This molecule is composed of four globular units, each of which is a chain of amino acids folded to form a 3-dimensional mass, two α -chains and two β -chains. Changes in the number or type of amino acids in the component molecules influences the shape (=conformation) and the function of the complete hemoglobin molecule. There are approximately 200 described variants (=mutations) in hemoglobin molecules. Naturally occurring variation in the β -chains can lead to the phenotype known as sickle cell anemia. In sickle cell anemia, there is a change in one amino acid of the β -chains compared to normal β -chains. This change makes sickle cell hemoglobin less efficient in carrying oxygen, and when oxygen concentration decreases, RBCs containing sickle cell hemoglobin change shape from biconcave to filamentous or sickle shaped. Sickle shaped RBCs do not move through blood vessels as easily as normal shaped RBCs (this RBC shape change gives the conditions its name). Presently, there is no cure for sickle cell anemia and it is potentially fatal, but this condition can be managed with medical attention. In the United States, approximately 80,000 African-Americans suffer from sickle-cell anemia. Approximately 8% of African-Americans (2.5 million) are carriers of the sickle-cell allele (heterozygous genotypes, also called sickle-cell trait). In sub-Saharan Africa, as much as 30% of some populations are sickle-cell allele carriers.

The change in an amino acid in the β -chain results from a change in the DNA (the gene) that codes for the hemoglobin β -chain. This β -chain gene can be symbolized as **Hb**, and has a normal form allele (**Hb^A**) and an altered form allele (**Hb^S**). The **Hb** gene is autosomal so every person has two copies of this gene, one from their mother and one from their father. The two copies of the **Hb** gene that each of us receives from our parents need not be the same. There are three possible genotypes: **Hb^AHb^A** which has the so called normal phenotype, **Hb^AHb^S** a heterozygote called sickle cell trait which is phenotypically similar to the normal phenotype but 50% of the hemoglobin molecules have the altered β -chains, and **Hb^SHb^S** which only produces the altered β -chains and is phenotypically sickle cell anemic. Since both the **Hb^A** and the **Hb^S** genes are expressed in the heterozygote, these alleles are co-dominant.

The difference in molecular structure between the **Hb^A** and the **Hb^S** hemoglobin β -chains makes identification of these proteins very simple. Electrophoresis is a technique for separating molecules in an electric field. Molecules with different electric charges will move at different rates when placed in an electric field. Molecules with a negative charge will be pulled toward a positive electrode, and the molecules with a strong negative charge will be pulled faster than molecules with a weak negative charge. The difference between the **Hb^A** and the **Hb^S** hemoglobin β -chains is a change from the amino acid glutamic acid to the amino acid valine. Glutamic acid has a negative charge but valine has no charge, so **Hb^S** hemoglobins have a weaker negative charge than do **Hb^A** hemoglobins. Consequently, **Hb^S** hemoglobins move more slowly toward a positive electrode than do **Hb^A** hemoglobins. This difference will permit us to perform electrophoresis on known and unknown hemoglobin samples and identify the genotypes of the unknowns.

Materials and Methods

Before attending the laboratory: Read about sickle cell anemia in your textbook. Answer questions 1-3 on the Hemoglobin Genetics Worksheet before your laboratory section meets (visiting the library to answer these questions is a good idea). You will complete questions 4-6 during the laboratory class and then submit the entire Worksheet to your laboratory instructor at the end of your laboratory class meeting.

You will place samples of hemoglobins in the wells of a 1.2% agarose gel. The entire gel (held in a running tray) is placed in a chamber containing a salt solution (Tris-glycine buffer, pH 9.2) that will conduct electricity. Electrical current flows through the agarose gel and the hemoglobin molecules in the wells move toward the positive electrode. The differential rate of migration by **Hb^A** and the **Hb^S** hemoglobins permits us to separate and identify these two types of hemoglobins. Note that in heterozygous **Hb^AHb^S** individuals, a given hemoglobin molecule will either have both β -chains of the normal form or both of the altered form, but not mixed in one hemoglobin molecule. Therefore, we can detect the **Hb^AHb^S** genotype by finding both **Hb^A** and the **Hb^S** hemoglobins in one individual.

We will provide you with known samples of **Hb^A** and **Hb^S** hemoglobins, and hemoglobin samples from a couple, labeled “father” and “mother” who wish to know the probability that they would produce children with sickle-cell anemia. Two student groups will share a single gel (four wells for each group).

Electrophoresis Procedures

Begin at least 1.5 hours before results are to be collected.

1. If gels have not been precast for you, follow the directions in the Appendix for preparing 1.2% agarose gels.
2. Once the agarose has cooled (30 minutes), or if already cooled, gently remove the comb by lifting at one end. Pulling the comb straight out will often pull the entire gel out of the tray, or rip the gel.
3. Lift the gel out of the casting tray by lifting the running tray and place the entire running tray with the gel on the central platform of the electrophoresis unit.
4. Gently pour running buffer (Tris-Glycine Buffer at pH 9.2) in the electrophoresis unit, but not directly on the gel. Fill until the gel is just covered with buffer (approximately 150ml).
5. Wear gloves and safety glasses when handling hemoglobin samples (these samples are a potential biological hazard). Using a micropipetter, transfer 10 μ l of each hemoglobin sample to a separate well in your agarose gel. Use a clean pipette tip for each sample so the samples are not cross contaminated. Eject used pipette tips in the beaker of bleach to sterilize before disposal. Record the order in which the samples are placed in the wells of your gel. Each group will use four of the eight wells and two groups will share one gel (see Figure 1). After all eight wells are filled with samples, place the clear plastic lid on the electrophoresis tray to keep the gel moist and to keep hands out. Orient the lid so the end of the gel with the sample wells is located closest to the negative (black) electrode.
6. Make sure the power supply switch is **OFF**. Connect the wires from the electrophoresis tray to the power supply, red to red and black to black. Switch the power supply on-hold, and adjust the voltage dial until the display reads 170 volts. Do not adjust the amperage dial.
7. Once the power is on, **DO NOT TOUCH THE WIRES OR OPEN THE TRAY**. The shock you might receive is not dangerous but is rather unpleasant.
8. Run the electrophoresis for at least one-hour. The bromophenol blue will migrate almost immediately, before the hemoglobin, and will move in the same direction as the hemoglobin samples. Stop the electrophoresis when the bromophenol blue is 0.25cm from the positive end of the gel.
9. When the electrophoresis is completed, turn the voltage to zero and switch the power supply off.
10. View the agarose gel in the electrophoresis tray through the clear cover. Your instructor will wipe condensation from the cover if necessary.

Acknowledgements

Based on a study published as *Hemoglobin Genetics and Mutation* in Blumer, L. Introduction to Biology, Laboratory Manual for BIO 101L. 1997. Wadsworth Publishing Co. Original protocol and text from Biology 100 Laboratory Manual, University of Michigan, 1984. Revisions made 1/2008 by L. Blumer.

Appendix

Preparation of electrophoresis gels (1.2% agarose)

For one gel:

25ml buffer + 0.3g powdered agarose

mix in a 100ml pyrex flask to form a suspension

heat on hot plate with a stirring bar (stir vigorously while heating)

agarose suspension should vigorously boil for 1 minute

remove from heat

cool 2-3 minutes at room temperature (until you can handle flask with bare hands)

agarose should be clear liquid

Pour into casing tray

Immediately insert comb at one end into liquid and push down until fully seated

Allow gel to cool 30 minutes (gel becomes cloudy as it cools)

4. How many different hemoglobin molecules does a person with sickle-cell anemia have in their red blood cells?

5. Draw a figure showing the results of the electrophoresis of the known and unknown hemoglobin extracts in this experiment.

6. Interpret the results you have shown above. What are the genotypes and phenotypes of the unknowns?