Laboratory 2 Biology 2250 Fall 2012
Protein Electrophoresis

Background

Read the background material given from Dr. Carr's lectures:

How Genes Work III: Protein Structure & Function

Molecular Basis of Heredity

Molecular Basis of Mutation

Enzyme proteins are made up of sequences of amino acids that represent the translated code of the nucleotide sequence of the gene. The amino acid sequence is the primary structure of the protein and single nucleotide substitutions can sometimes result in an amino acid substitution.

An enzyme protein with an amino acid substitution can be detected if this substitution results in a change in the net charge of the protein molecule. Protein molecules with different net charges will move at different rates in an electric field. Protein electrophoresis separates proteins in an electric field and uses staining methods that take advantage of the specificity of an enzyme for its substrate to localize the enzyme protein as bands on a gel.

Allozymes are proteins with minor changes in electric charge that can be detected using **protein electrophoresis**. An individual can be a homozygote with two copies of the same allozyme or a heterozygote consisting of two different allozymes.

This laboratory consists of the three exercises listed below. **Please review all of the exercises before your laboratory period.**

Exercises:

1. **PROTEIN ELECTROPHORESIS**

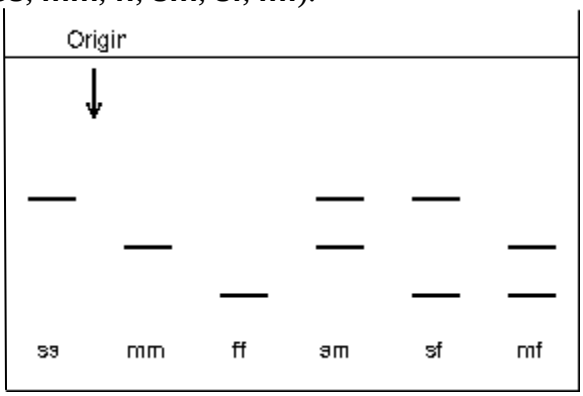
Many or most of the organisms of interest to geneticists lack the detailed genetic map and wealth of morphological differences found in organisms such as the fruit fly, *Drosophila spp*. Genetic studies of such species can instead make use of the many genes that code for enzymatic proteins. The different **alleles** of such genes code for different forms of the same enzyme (**allozymes**) which, due to differences in amino acid composition that affect the electric charge of the enzyme, migrate at different rates in an electric field. The allozymes can then be visualized as bands on a gel (an **electropherogram**: see Figure 1) by the use of histochemical staining techniques that take advantage of the specificity of enzymes for their substrate.

Thus, the **genotype** of alleles at a locus in an individual can be analyzed as a **phenotype** of allozymes that differ in electrophoretic mobility. For example, say samples tested for the locus ***PGM*** for the enzyme **phosphoglucomutase** had three alleles. They could be called ***PGM*****s**, ***PGM*****m** & ***PGM*****f** that produce "*slow*", "*medium*" and "*fast*" allozymes, respectively. [Be careful to distinguish among the *locus*, its *alleles* and the *allozymes* the alleles produce: for brevity, the alleles and their allozymes are often referred to interchangeably with the same symbols]. For a gene with three alleles, there are six possible genotypes and six phenotypic patterns: an individual may be a homozygote (**ss**, **mm** or **ff**) or a heterozygote (**sm**, **sf** or **mf**). The alleles are expressed **codominantly**: homozygotes show a single band corresponding to a single allele, heterozygotes show two bands corresponding to both alleles. Electrophoretic alleles are inherited in a Mendelian fashion: a cross between two heterozygotes that show the **sf** pattern will produce the expected ratio:

(**sf** x **sf** => 1 **ss** : 2 **sf** : 1 **ff**)

Because the enzymatic properties of proteins are constant among different species, the same histochemical staining techniques can be used in a wide variety of organisms, including plants, animals, and microorganisms. Protein electrophoresis is widely used in genetics to analyze the progeny of particular genetic crosses, or to measure the extent of genetic variation in natural populations (the latter application will be discussed in more detail in Bio2900).

Figure 1 is a diagrammatic representation of an electrophoretic gel stained to reveal enzyme protein bands. The **Origin** is where each sample is loaded, and the arrow indicates the direction of migration in an electric field. The protein products corresponding to each of the three alleles are named according to their relative mobility [slow (**s**), medium (**m**), and fast (**f**)], which gives six possible gel phenotypes (**ss**, **mm**, **ff**, **sm**, **sf**, **mf**).



This exercise will illustrate the method for extracting enzymes, running enzymes in an electric field (protein electrophoresis) and staining to visualize differences in electrophoretic mobility of the alleles. Protein electrophoresis will be conducted on a plant species called *Barbarea vulgaris*. (For pictures, click HERE.) Two enzyme stains will be used: one specific for the enzyme **phosphoglucose mutase** (***PGM***) and one stain specific for the enzyme **phosphoglucose isomerase** (***PGI***). You will be provided with tissue samples for you to grind into a homogenate. These are examples of various possible genotypes from different local sites, not the result of a particular cross. From the genotypes obtained, allele frequencies will be determined for each site and then the sites will be compared for similarities and/or differences.

Each of the gel plates you set up will have the same samples, but each will be stained to look for a different enzyme.

Materials:

Tissue samples*:* leaves from *Barbarea vulgaris*
grinding well plate
100ml fine sand
spatula
pasteur pipettes
P-20 or P-200 micropipettor & tips
forceps
2-50 ml beakers
2-250 ml beakers
2 pestles
2 cellulose acetate plates (in 500 ml beaker with buffer)
blotting paper
well plate
applicator base
comb applicator
glass plate
electrophoresis tank
power supply
Bromophenol Blue tracking dye
TRIS – glycine Buffer
Grinding Buffer (Tris HCl with mercaptoethanol)
Wax pencils
Hot Water Bath (70 ˚C)
Razor blades

Procedure: (work in groups of 2-4)

To make the homogenates of *Barbarea vulgaris:*

1. Obtain 10 leaves from the sampling site bag assigned to your group by the Lab Instructor. Half the class will be assigned leaves from one location and the other half will be assigned leaves from another location. **Important: On page 6, make sure you record the sampling site location indicated on the sampling bags for both your site and the other site.** Tearing small amounts off of each leaf (as demonstrated by the lab staff), place the pieces in wells # 1-10 of the grinding well plate.

2. Using the fine spatula provided, add a *small* amount of fine sand to each well. Next, add 2-3 drops of grinding buffer (IMPORTANT!!! It's the **Tris HCl with mercaptoethanol** in it) to the plant material.

3. Using the blue pestles, **and rinsing the pestles with tap water (in a 250ml beaker) between samples**, grind up the pieces of leaves in each well. This is the *Barbarea vulgaris* homogenate. Have your homogenate checked by the Lab Staff.

Transferring and loading the 2 gel plates:

1. Using the correctly calibrated micropipettors, pipette 15 µl of homogenate of each sample into separate wells (##1-10) of the small well plate. **Use a new tip for each sample and slice the very end of the tip off with a razorblade!** To well 11, add 10 µl of Tracking dye in order for you to determine if the electrophoresis procedure is working properly.

2. Use forceps to gently remove one of your two gel plates from the buffer solution. **Remove excess fluid by blotting with the blotting paper.** Handle by the edges and try not to scratch the gel. **Work quickly: don't let the gel plate become dry**.

3. Position the gel plate, shiny side down, on the applicator base, lining it up with the penciled outline on the base. [A drop of water on the base will keep the gel plate from slipping around]. Using the comb applicator, transfer the samples from the well plate to the gel plates, gently applying the samples about 1 cm from the origin end of the gel plate. **BE CAREFUL**: **DO NOT SLAM THE COMB APPLICATOR DOWN ONTO THE GEL PLATE –THE TEETH ARE VERY FRAGILE!**

Make a second application to the same plate to ensure enough sample has been transferred.

4. Place the gel plate sample (dull) side down into the running tank with the origin on the same side as the black (negative) terminal.

5. Repeat step #2- 4 for the next gel plate. Using a Pasteur pipette, add some of the buffer in the tank to the edges of the gel plates. **Get an Instructor to check your plates**.

6. Close the tank lid, and turn on the power. The power supplies need to run at ~3-4 mA (no more than 4). However, if your group is sharing the power supply with another group, run it at ~8mA.

***Clean all applicator equipment used so far NOW by rinsing well with tap water!!**

Check the tank after 5 minutes to be certain that the gel is running (& in the right direction). Let the electrophoresis continue for about 25 minutes: the blue tracking dye should be visible about 1 cm from the finish of the gel plate.

7. While the gels are running, start to mix the following stain solutions in separate, **labelled** 50 ml beakers. **(Wear gloves while mixing the stains and discard the gloves, along with the gels, in the Biohazard bags when you are finished with them).** Make sure you see the notes about ingredients marked with "*".

Stain recipes:

Stain for PGM (Phosphoglucose mutase):
Tris HCl pH 8.0 10 drops
NAD 5 drops
Glucose-l-phosphate 5 drops
MTT 5 drops
*****PMS 5 drops
*****G6PDH 10 microlitres (µl)
******Agar ~ 3 ml

Stain for PGI (Phosphoglucose isomerase):
Tris HCl pH 8.0 10 drops
NAD 5 drops
Fructose-6-phosphate 5 drops
MTT 5 drops
*****PMS 5 drops
*****G6PDH 10 microlitres (µl)
******Agar ~ 3 ml

*****Add just before adding the stain to gel plate.
****** The Lab Instructor will add the agar once you've brought your beakers of stains AND the gel plates up to the hot water bath in Step 9 below.

8. After the gels have been running approximately 25 minutes, turn off the power. Remove the gel plates and place them, dull side up, at least 2 inches apart on the glass plate.

9. Finish mixing the stains. Bring the stains and the gel plates on the glass plate up to the hot after bath where the agar will be added by the Lab Instructor. The agar must be warm enough to be poured; if too cold it will solidify before it can cover the gel plate.

10. The stain will be poured, starting in the centre of each gel plate, so that it completely covers the corresponding plate.

The PGM & PGI plate should show dark bands in approximately 1-2 minutes.

Results:
Each gel plate has the same samples in the same numbered wells. The plates have been stained differently to determine the genotype of a specific enzyme. The bands will have migrated different distances and the banding patterns will be different for each enzyme. Each loci for each enzyme has 4 alleles (corresponding to **1, 2, 3 and 4**), with Allele 1 the slowest in terms of relative mobility and 4 the fastest. Note, however, that not all alleles may be present in the samples used.

After you have determined the genotypes of the PGM and PGI allozymes for each leaf sample for your group, record your findings for both PGI and PGM in Question 1B. Also, record your results for PGI in the posted class table, which will be used for some of the questions.

While you are waiting for the gels to run, complete exercises 2 and 3. To access these exercises, go to www.mun.ca/biology/dinnes/B2250/Lab2/Lab2.htm and click on their links for more information on each exercise.

Exercise 2. Scoring enzyme banding patterns

Exercise 3. Quaternary Structure: Monomeric and Dimeric Proteins.
Name_____
MUN # _____

Lab Slot _____

Site Location of YOUR Protein Electrophoresis Samples: _____
Site Location of the OTHER Protein Electrophoresis Samples: _____

You will answer the following questions **in lab** and hand **these pages in** at the **end** of the lab period.

1. Protein Electrophoresis:

0.5 **A.** What are allozymes? Are PGI and PGM allozymes of each other? Why or why not?

_____
_____
_____

0.25 **B.** List the allozyme genotypes scored for the *Barbarea vulgaris* individuals run for PGI and PGM in YOUR samples. **Also fill in the data sheet provided by the Lab instructor for your samples as well**.

PGI PGM

Table 1: Class Results of the Genotypes of Allozymes of PGI and PGM from 10 *Barbarea vulgaris* leaves from Site 1

RESULTS FROM SITE 1 - _____							
	Genotypes of PGI allozymes				Genotypes of PGM allozymes		
Leaf #	Group 1	Group 2	Group 3	Leaf #	Group 1	Group 2	Group 3
1				1			
2				2			
3				3			
4				4			
5				5			
6				6			
7				7			
8				8			
9				9			
10				10			

Table 2: Class Results of the Genotypes of Allozymes of PGI and PGM from 10 *Barbarea vulgaris* leaves from Site 2.

RESULTS FROM SITE 2 - _____							
	Genotypes of PGI allozymes				Genotypes of PGM allozymes		
Leaf #	Group 1	Group 2	Group 3	Leaf #	Group 1	Group 2	Group 3
1				1			
2				2			
3				3			
4				4			
5				5			
6				6			
7				7			
8				8			
9				9			
10				10			

1.0 **C.** Based on the **class data**, calculate the allele frequencies/ percentages **of the alleles present**, for **PGI ONLY** for each sample site, showing all calculations:

SITE 1 SITE 2

0.5 **D.** Compare Site 1's allele frequencies with Site 2's. Offer an explanation as to why the similarities and/or differences in allele frequencies obtained between the two sites may have occurred.

_____
_____
_____
_____
_____
_____

E. (**NOTE: Complete Exercise 2 before answering this question**)

0.25 How could you determine from the results of a gel electrophoresis if PGI and PGM are monomeric or dimeric?

_____
_____
_____
_____

2. Scoring Enzyme Banding Patterns:

In order to help you score the bands: make note of the following information:

Gel 1 – there are 3 allozymes (a to c), with a on the top row, c on bottom row

Gel 2 - there are 4 allozymes (a to d), with a on the top row, d on the bottom row

Gel 3 – the naming of this gel is upside down. Ignore the top band. There are 2 allozymes (this is a dimeric enzyme so when it is heterozygous there is an intermediate band) (a to b), with b on the top row, a on the bottom row).

Gel 4 - there are 5 allozymes (this is a dimeric enzyme so when it is heterozygous there is an intermediate band) (a to e), with a on the top row, e on the bottom row. The first 8 enzymes are: cc cd bb ac cc cd ac ce. **Use this to help you work out the rest**.

A. Give the allozyme genotype for the individuals for the 4 gels in order (L to R)

1.0 Gel 1: _____

Gel 2: _____

Gel 3: _____

Gel 4: cc cd bb ac cc cd ac ce _____

3. Quaternary Structure: Monomeric and Dimeric Proteins:

0.5 **A.** i. Describe how homozygotes and heterozygotes differ from one another in terms of the numbers of the allozymes in each and whether the allozymes present are the same or different from each other.

_____
_____
_____
_____
_____
_____
_____
_____

0.5 **B**. In **monomeric** enzymes, describe how heterozygotes and homozygotes differ from one another in terms of the number of bands or peaks seen and the intensity of each band (as seen by the heights of the peaks).

_____
_____
_____
_____
_____

0.5 **C**. In **dimeric** enzymes, describe how heterozygotes and homozygotes differ from one another in terms of the number of bands or peaks seen and the intensity of each band (as seen by the heights of the peaks).

_____
_____
_____
_____
_____