Molecular Biology

Lab 7

Protein Gel Electrophoresis

SDS-PAGE

 More GFP!

A few weeks ago you were introduced to DNA agarose gel electrophoresis. Electrophoresis of proteins follows the same basic principles. Proteins of different sizes and charge can be separated by migration through a porous gel placed in an electrical field. Proteins are loaded into a well at the negative end of the field and allowed to migrate toward the positive end.

Proteins are not uniformly negatively charged. Those with an abundance of negatively charged amino acids are, but proteins can be positively charged (recall histones?) or neutral. To compensate, proteins are typically loaded into a gel in a buffer, which contains the detergent, sodium dodecylsulfate (SDS). This negatively charged compound, binds to proteins and gives all proteins a negative charge. This allows them to migrate away from the wells and to enter the gel, no matter what their natural charge.

DNA fragments can vary in length, which can be detected using electrophoresis. However, DNA of any source, of any length assumes a consistent shape---the double helix. So electrophoretic migration distance reflects DNA fragment length alone. (One exception you have experienced is *circular* DNA, which can become supercoiled and more compact, or relaxed and bulkier than linear fragments of the same size.) Proteins, on the other hand can assume a wide variety of folded shapes, all of which affect migration through the gel matrix. For example, a very large protein, in terms of amino acid number, might fold into a fairly compact blob and migrate quite quickly, and a protein made of fewer amino acids may be more long and floppy, and move quite slowly through the gel. So migration does not always reflect absolute size/molecular weight. In any case, agarose is not a suitable gel for fine separation of proteins. Polyacrylamide is typically used for protein electrophoresis. Protein electrophoresis is commonly called SDS-PAGE (SDS polyacrylamide gel electrophoresis).

Protein gel electrophoresis can be varied in a number of ways, depending on what type of analysis you need to do. For example, if you want to determine absolute size/molecular weight of a protein a number of methods can be used to unfold or denature a protein. One of the easiest is to heat the samples to near boiling temperature and to include in the electrophoresis buffer components that keep the proteins denatured. Denaturing SDS-PAGE completely unfolds proteins, making them non-functional. So if the goal is to isolate a functional protein, this is not the method to use.

 Polyacrylamide gels can be made in different ways to accommodate separation of proteins of size ranges. The gel we use today will actually vary in gel percentage in different parts of the gel. This will allow resolution of proteins that span a large range of molecular weights.

Today we will separate the proteins of pGLO transformed *E. coli* using SDS-PAGE. Many aspects of the lab will help in understanding characteristics of GFP and of proteins in general.

 Protocol:

Materials

LB-A plate of pGLO-transformed *E. coli*

LB-A-A plate of pGLO-transformed *E. coli*

Tube 4 from pGLO protein purification lab

Sterile loops

Tube Laemmli buffer +DTT

Pre-cast polyacrylamide gel (4%-20%)

Vertical gel apparatus and power supply

10X electrophoresis buffer (Tris-Glycine-SDS)/TGS

UV light

Comassie gel stain

Protein standards

Hot plate/beaker to heat to 95 degrees C.

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1. Begin heating a beaker of water on the hot plate. Bring the water to a near-boil.
2. Make a liter of 1X TGS from the 10X concentrate
3. Label 4 tubes for your pGLO transformed bacteria
	1. LB-A transformants
	2. LB- A transformants (heated)
	3. LB- A-A transformants
	4. LB- A-A transformants (heated)
4. Label 1 tube for your column purified GFP
5. Pipette 300 l \*Laemmli Buffer into tubes **a** and **c**.
6. Take one healthy loopfull of bacteria from the LB-A plate and dispense the bacteria into the Laemmli buffer in tube **a** by twirling the loop between your finger and thumb. You should see the blob of bacteria on the loop and then all the bacteria should be shaken off the loop into the buffer.
7. Transfer 150 l of the bacterial mix to tube **b**.
8. Repeat with bacteria from the LB-A-A plate. Take one healthy loopfull of bacteria from the LB-A-A plate and dispense the bacteria into the Laemmli buffer in tube **c** by twirling the loop between your finger and thumb.
9. Transfer 150 l of the bacterial mix to tube **d**.
10. Transfer 100 l of your HIC-affinity column purified GFP to the final tube.
11. Incubate tubes b and d in the near boiling water for 5 minutes. Allow to cool.
12. Assemble the pre-cast acrylamide gels in one apparatus.
13. Fill the inner chamber with 1X TGS
14. Fill the outer chamber ~2/3 full
15. Using about 50 l oad the gel according to a pre-outlined scheme. Include lanes for protein standards.
16. Run for 30 minutes at 200v.
17. While the gel runs, make some hypotheses about what you may see on the gel after it has run. Check the gel often.

After electrophoresis.

1. Keeping the gel in its frame, look at the gel. Note the separation of the protein standards---why do we use these standards? Measure the migration of as many standards as possible.
2. Look for GFP among the proteins you added to the gel. Measure the migration of GFP from the bottom of the loading well.
3. Approximately how big is GFP?
4. Carefully disassemble the gel and place into a plastic tray filled with distilled water. Slosh the gel for 5 minutes.
5. Stabilize the gel with a gloved finger and pour off water. Repeat the water rinse 2 more times.
6. Pour off all the water. Cover the gel with the Coommasie Blue protein stain. I will rock the gel with stain on it overnight and we will examine the gels tomorrow.
7. Form some hypotheses about what you will see.