Experiment #6b
Protein Analysis via SDS Electrophoresis

Introduction

Electrophoretic techniques have become principal tools for characterizing macromolecules and for assaying their homogeneity. The method is based on the fact that molecules such as DNA, RNA and proteins possess a charge and therefore are able to move when placed in an electric field.

If a molecule of charge **q** is placed in an electric field, a force **F** is exerted upon it, which depends on the charge possessed by the molecule and the strength of the electric field. Mathematically,

$$F = q.E / d \qquad (1)$$

Where **E** is the potential difference and **d** is the distance; the term **E/d** is often referred to as the field strength. The movement of a macromolecule through a viscous medium in response to this electric field is opposed by a drag force,

$$F = 6 \pi \eta r v \qquad (2)$$

where **r** is the radius of the spherical molecule, η is the viscosity and **v** is the velocity at which the molecule is moving. Combining these two equations, it can be seen that the velocity is proportional to the field strength and charge on the molecule, and is inversely proportional to the size of the molecule and the solution viscosity.

$$v = E.q / (6 \pi \eta r d) \qquad (3)$$

Electrophoresis of proteins is usually carried out using polymerized acrylamide, crosslinked by a small amount of *bis* -acrylamide, as a supporting medium. The pores formed by the acrylamide matrix typically vary between 0.8 and 4.0 nm and movement of molecules through the gel is controlled to some extent by the ability of the molecule to partition within the pore; mobility within the gel is therefore a function of both molecular size and net charge.

Acrylamide electrophoresis of proteins is particularly useful when carried out in the presence of the anionic detergent sodium dodecyl sulfate (SDS). Most proteins denature in the presence of SDS to form extended, random coil structures; reducing agents are also added to cleave any disulphide bonds, thus forming flexible coils with a more-or-less constant charge-to-length ratio. Because of this, proteins can be electrophoretically separated based on random-coil size, or, their apparent molecular weight. Further, an empirical relationship exists between the mobility of a protein on SDS electrophoresis and its molecular weight; a plot of **log(MW) against mobility** is generally **linear** for a set of known molecular weight proteins. Electrophoresis of an unknown in a lane adjacent to a series of standards can therefore be used to estimate the molecular weight of the unknown protein; the presence or absence of other bands in the gel can also be used to estimate homogeneity with respect to molecular size.

In this experiment, you will perform SDS electrophoresis on the purified β-lactamase Tem-1. Based on electrophoretic mobility, you will be able to estimate the molecular weight of your protein and its homogeneity with respect to molecular size.

Experiment

1. A 12% acrylamide gel should be prerun in 1x SDS buffer at 130-150 volts. Make sure that the gel is viable by visual inspection. Do not forget to remove the white strip of tape on the back of the gel if it has not already been done. The back of the gel cassette should always face away from the inner part of the electrophoresis apparatus. Your TA will show you the proper setup.

2. Obtain Tem-1 enzyme and 2x loading dye from your TA. Pipette 15 μL of 2x loading dye into two microfuge tubes. Next, add 10 and 15 μL respectively of Tem-1 enzyme to each tube. To the tube in which 10 μL of enzyme was added, add 5 μL of distilled water.

3. While the gel is prerunnig for about 15 minutes you should add 2 μL of mercaptoethanol to each sample. Be sure to do this in the hood, and then boil the samples for 5 minutes. Note that you will be sharing a gel with other groups to save space. Carefully add

your sample to the well and record the proper position of each sample to avoid confusion with other groups. Make sure that a protein ladder sample is loaded on each gel to serve as a reference.

4. Once the gel is running it will take about an hour to completely separate the protein bands. The power supply can be shut off and the gel removed once the dye bands are near the bottom. Cautiously remove the gel cassette from the apparatus, and follow the demonstration by your TA as to how to remove the actual acrylamide gel from the gel holder.

5. After the gel has been carefully removed, you may place it in an empty pipette tip box filled with 15 ml of staining solution and 15 ml of 20% acetic acid. The gel should stain for 25 minutes while being gently shaken. After this time, the staining solution should be removed, and the destaining (6:1:3 water:acetic acid:methanol) solution should be added to visualize the protein bands on the gel. It should also be shaken during this time and the solution changed periodically to facilitate efficient gel destaining.

Data Analysis

From the gel itself or the picture provided by your TA, measure the mobility of your sample, defined as the distance from the origin to your sample divided by the distance from the origin to the tracking dye. Likewise, determine values for the mobilities of the standard proteins that were run. Using a computer program, enter the dataset for the protein standard curve into two columns as log(MW) versus mobility. Plot the dataset and fit the plot with a linear regression. Using the value determined for the mobility of your protein, calculate the molecular weight of Tem-1. If you have several protein bands in your sample, Tem-1 has a molecular weight of 31,000 Daltons.