Chemistry 103

Lab 5: Protein electrophoresis

Objective: To use the technique of protein electrophoresis to determine the length(s) of the component(s) of the fungal protein, alpha amylase.

Protein electrophoresis is the technique used to separate and characterize proteins and polypeptides by molar mass. Molar masses (molecular weights is a synonymous term) are measured in g/mol, unless you’re a biologist in which case they are measured in Daltons (D), which is the same as a g/mol. Since proteins are quite large, their masses are more appropriately measured in kilodaltons (kD).

The separation is accomplished by exploiting two physical effects: first, that if you have a mesh, it takes longer for big things to get through the mesh than little things; and, second, that proteins may be electrically charged so that they will move in the presence of an external electrical field.

The mesh is provided by cross-linking the deadly neurotoxin acrylamide with bis-acrylamide to form a polyacrylamide gel (incidentally rendering the neurotoxicity harmless). The external electrical field is provided by the Protean 3 gel electrophoresis box, pictured below. You will load protein samples into the “wells” of the gel near the top, and allow the proteins to be drawn down toward the bottom of the gel by the electrical field.

Materials

• Mini-Protean 3 cell (protein gel electrophoresis tank)
• Power supply and cords
• Practice loading sample
• Bovine serum albumin (BSA)
• β-amylase
• soybean trypsin inhibitor
• myoglobin
• Kaleidoscope prestained protein standards
• Two large weigh boats for gel staining and destaining
• Constant temperature (95°C) water bath
• Protein Instastain sheet
• 50% methanol/10% acetic acid in distilled water solution (fixing solution)
• Laemmli sample buffer (add 50 μL β-mercaptoethanol to 950 μL buffer prior to lab)
• Precast Tris-HCl polyacrylamide gel
• 1x Tris-glycine buffer for running the gel

Safety issues: The buffers, the acrylamide and the β-mercaptoethanol are all toxic; please use goggles throughout the lab, and disposable gloves when handling the stained gel.
Procedure

Preparation of the protein samples
1. The protein samples are all “native” proteins; that is, they have not been denatured and thus are not linear in shape. Each group will be assigned one of the native proteins to denature; obtain 10 μL of that protein and put it in a microcentrifuge tube. **Label the tube with the name of the protein.**

2. To denature the protein, add to your centrifuge tube 10 μL of Laemmli buffer (with β-mercaptoethanol already added) to the sample. Flick the tube to mix the sample into the reagents.

3. Heat the tube for 5 minutes at 95°C in the water bath.

Preparation of the protein electrophoresis unit
Remove the gel cassette sandwich from its wrapper. If present, remove the tape along its bottom (leave in the comb at the top for now).

Place the gel cassette sandwich into the electrode assembly, with the short plate facing inward.

Slide the gel cassette sandwich and electrode assembly into the clamping frame (you will have to use a plastic plate to block the other side if you have only one sandwich.

Press down the electrode assembly while closing the two cam levers of the clamping frame. Lower the inner chamber into the mini-tank.

Fig. 5. Mini-PROTEAN 3 assembly.
Running the gel electrophoresis
Fill the inner chamber with about 125 mL of running buffer until the level of buffer reaches halfway between the tops of the taller and shorter plates of the gel cassettes.

Add about 200 mL of running buffer to the mini-tank’s lower buffer chamber.

Carefully remove the comb to expose the wells in the gel.

Practice loading the gel by using the 10 µL micropipetter (with a fresh disposable tip) to pipet 10 µL of the “practice sample loading” solution into Lane 1. Several students can take turns loading this sample into the same well (Lane 1). Keep an eye on how the sample is slightly denser than the running buffer and how the sample therefore “settles” to the bottom of the well. Load the sample slowly to allow it to settle evenly on the bottom of the well. Be careful not to puncture the bottom of the well with the micropipette tip. Also note how jerky motions with the tip of the micropipetter will upset the settling of the sample, and how a smooth motion to withdraw the tip as the last of the sample is expelled is the ideal loading strategy.

When you are confident, load 10 µL of each sample into different wells with the micropipette (clearly, with a fresh disposable tip each time). Start with Lane 2 and move out from there. Have your teammates write down which lane has which sample to avoid confusion. Ultimately, you should have loaded (not including Lane 1) seven lanes (the Kaleidoscope prestained standards (which should be in Lane 2), the four separate native protein samples, the mixture sample, and the denatured protein sample).
Place the lid on the mini-tank. Make sure to align the color-coded banana plugs and jacks. The correct orientation is made by matching the jacks on the lid with the banana plugs on the electrode assembly.

Insert the electrical leads into a suitable power supply. **Make sure you get the polarity of the plugs oriented correctly!**

Turn on the power switch to the power supply and adjust the voltage to 125 volts. Run the gel for 60 minutes or so, until you see a good separation in the colors of the Kaleidoscope standards.

After the electrophoresis is complete, turn off the power supply and disconnect the leads.

**Staining the gel**

Carefully remove the short plate from the gel (use gloves) by wedging the plates apart with a spatula. **Do not tear the gel.**

To a large weigh boat, pour enough 50% methanol/10% acetic acid (fixing) solution to cover the gel. Carefully slide the gel off of the tall plate into the fixing solution. Allow the gel to fix for 10 minutes with gentle agitation.

Place the gel on plastic wrap on a flat surface. Pipet about 1 to 2 mL of fixing solution on top of the gel, and place the Protein Instastain sheet, blue side down, over the top of the gel. Press the sheet firmly with the back of your hand but avoid tearing the gel underneath. Place one of the glass plates on top of the Instastain sheet and a 500 gram weight on top of the glass plate (a large beaker from your drawer will do).

Allow gel to stain for 20 minutes.

Carefully remove the weight, glass plate and Instastain sheet (put this in the solid waste container in the hood) from the top of the gel.

Place the gel back into the fixing solution. When the surrounding solution becomes blue, pour off the fixing solution into another container (which will later be poured into aqueous waste). Add fresh fixing solution and continue discarding blue fixing solution. Stop when the fixing solution no longer turns blue — this should take about 10 minutes.

Place the gel in the gel photography box, if available. Take a photograph of the gel under white light illumination.

**Data**

Sketch the gel as best as you can, either from the gel itself or attach the photograph to your lab book. The numbers you will be needing are the distance
each “band” (which represents a protein) is away from the well, so both the band and the well should be clearly visible on your sketch.

Make a table to list the different distances as follows:

<table>
<thead>
<tr>
<th>Lane 2 (Kaleidoscope standards) band migration distances (mm)</th>
<th>(list separately the distances of the different bands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 3 (native bovine serum albumin) band migration distances (mm)</td>
<td>(probably only one band distance here)</td>
</tr>
<tr>
<td>Lane 4 (beta amylase) band migration distances (mm)</td>
<td></td>
</tr>
<tr>
<td>Etcetera...</td>
<td></td>
</tr>
</tbody>
</table>

Set up another table to determine the correlation between band migration distance and protein molecular weight as follows:

<table>
<thead>
<tr>
<th>Kaleidoscope standard migration distance (mm)</th>
<th>Protein molecular weight (kD)</th>
<th>Protein name</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>myosin</td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>β-galactosidase</td>
<td></td>
</tr>
<tr>
<td>82.5</td>
<td>bovine serum albumin</td>
<td></td>
</tr>
<tr>
<td>40.5</td>
<td>carbonic anhydrase</td>
<td></td>
</tr>
<tr>
<td>31.9</td>
<td>soybean trypsin inhibitor</td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>lysozyme</td>
<td></td>
</tr>
<tr>
<td>6.97</td>
<td>aprotinin</td>
<td></td>
</tr>
</tbody>
</table>

In exercise 6, you will figure out how to determine the molecular weight(s) of the various native proteins and your denatured protein.

**Questions**

1. What is the **purpose** of heating the tube for 5 minutes at 95°C? Yeah, it’s to denature the protein, but what does that really mean at the **molecular** level?

2. Staining allows the “invisible” protein to be visualized. Why is **destaining** necessary?

3. Why might you **not** want to wait a couple of days to image your gel results? Hint: will the protein stay put in the area it ended up when the electrical field was turned off?
4. Beta-amylase, albumin and trypsin inhibitor are globular proteins with similar pIs and with gross conformations that are roughly spherical or ellipsoidal. What is the primary reason, then, for the differences in their electrophoretic mobility?

Discussion of results and conclusion – write a paragraph (or provide a table) for each of the following questions.

• What is the molecular weight (in kD) of the native and the denatured versions of your protein, according to the calibration provided by the Kaleidoscope standards?

• Was there a difference in the electrophoretic behavior of the native and the denatured versions of the same protein (each group might have a different answer to this question)? Describe the difference and determine some possible causes.

To turn in: I merely require the sketch or photo of your gel, and photocopies of: the two data tables, the answers to the questions and the “discussion of results and conclusion”.