Protein Electrophoresis

A typical eukaryotic cell contains tens of thousands of different proteins, each involved in specific cellular processes. The heat-shock proteins were originally identified because they are produced in large quantities when cells are subject to heat-induced stress. Because subsequent studies have demonstrated that they are produced in response to a variety of stresses, these proteins are now often referred to as stress proteins. It is important to recognize that stress proteins have essential roles in non-stressed cells, where they function normally in the synthesis, transport and folding of non-heat-shock proteins.

The stress proteins are produced to protect the cell from situations that would lead to irreversible cell damage and ultimately to cell death. Under conditions of stress, the genes encoding the heat-shock proteins are activated and large amounts of the proteins are produced. When the stress is relieved, the level of these proteins returns to normal. These changes can be observed by examining the protein content of a cell using polyacrylamide gel electrophoresis (PAGE). In Drosophila, the most prevalent heat-shock proteins belong to the hsp70 family, with molecular weights of around 70,000 daltons. The activation of heat-shock genes can be observed at the DNA level, by examining the giant polytene chromosomes of Drosophila. Enhanced gene expression is correlated with chromosomal “puffing”, which is characteristic of certain regions of the chromosomes that are being actively transcribed.

The examination of the proteins in a cell requires several steps, including isolation, separation, and visualization. Isolation is accomplished by homogenizing a tissue sample in an appropriate buffer. Separation and visualization are accomplished by electrophoresis in polyacrylamide gels under denaturing conditions. Before loading the sample on the gel, the protein is denatured using a combination of a detergent, such as SDS; a reducing agent, such as mercaptoethanol; and heat. This ensures dissociation of the individual polypeptide subunits that are characteristic of many proteins.

There are two objectives of this exercise: (1) To isolate proteins from Drosophila cultures that have been subjected to heat shock. (2) To analyze these proteins using polyacrylamide gel electrophoresis so that a comparison can be made between heat-shocked and non-heat shocked samples.
Procedures

IMPORTANT NOTES

• During protein isolation it is important to keep the sample on ice at all times.

• Before beginning, make sure that the centrifuge and rotor are pre-cooled to 4°C. It is important that all of the class’s samples are spun at the same time so start the isolation only when you are told to do so.

• If the isolation and electrophoresis procedures will be completed in one day, make sure that you have a heating block ready at 100°C before starting. Also, make sure that the gel is pre-run for at least 15 minutes before the samples are loaded.

A. Protein Isolation

1. Obtain two frozen *Drosophila* samples, one labeled “HS” (heat shock) the other “C” (non-heat shock control). Different cultures were heat-shocked at different temperatures so make sure to note which temperature was used with your sample (e.g., HS-37; HS-42).

2. Work with one of the sample tubes at a time, keeping the other on ice. Using a micropipettor, add 50 μl ice-cold homogenization buffer to the sample. Quickly homogenize the samples on ice until no tissue is visible.

3. Transfer the tubes to a pre-cooled centrifuge and spin at 10,000 xg for 10 minutes. While the tubes are spinning, label two fresh screw-cap tubes “HS” and “C”, and place on ice.

4. Remove the tubes from the rotor and immediately place them on ice. Using a micropipettor, transfer 25 μl of each supernatant to its respective cold, fresh tubes. Avoid the pellet and any material floating on the top of the supernatant. The material on top is the lipid fraction of the homogenate and it is critical that none of this be transferred with the sample. The lipids sometimes adhere to the outside of the pipet tip; if that happens, try not to submerge the tip as you transfer the supernatant to the fresh tube.

If the samples are to be loaded on the gel immediately, proceed directly to section B. If the samples will be analyzed later, store them at –20°C and thaw on ice before proceeding.

B. Electrophoresis

Make sure that the gel is pre-run at 100 volts for at least 15 minutes before the samples are loaded.

1. Add 5 μl of loading buffer to the contents of each tube; place in a heating block set at 100 °C and incubate for 3 minutes. Place the tubes at room temperature to cool before loading.
To demonstrate the proper loading procedure, your instructor will add 10 μl of a size standard to the first well of each gel. The size standard (“protein ladder”) contains bands of between 10,000 and 200,000 daltons. *Do not heat the protein ladder.*

2. Carefully load 25 μl of each denatured sample in adjacent empty wells. Load the sample slowly in a steady stream so that it has time to sink to the bottom of the well. Make sure you record the lane number and the identity of your samples, as well as their positions relative to the size standard used.

3. After all of the samples in the class have been loaded, carefully connect the leads to the electrophoresis chamber and run the gel at 100 volts until the loading dye has migrated completely off of the gel (about 1 hour). Note the time and continue running the gel for an additional 30 minutes.

4. Turn off the power supply and disconnect the power cords from the chamber. Your instructor will show you how to remove the gel from the plates and stain it.

Staining and destaining take a minimum of two hours and are preferably done overnight. Since the gels are stable indefinitely, it may be convenient to look at the results on another day.

**Points to Ponder (as you write your report)**

The sample buffer that is added to the protein before loading on the gel contains a detergent (SDS) and a reducing agent (mercaptoethanol). What is the purpose of these two compounds?

Why are the protein samples heated before loading on the gel?

The gel you are using has a concentration of 12.5% acrylamide. If you wanted to resolve proteins larger than the 70,000 dalton heat-shock proteins, would you use a higher or lower concentration of acrylamide?

Would you expect the other (non-heat shock) proteins in a cell to be produced at the same, reduced, or elevated levels during heat shock when compared to their normal levels? What did you observe when comparing the heat-shock and control protein samples?

What is the relationship between the normal function of heat-shock proteins in a cell and their function during the stress response?

The heat-shock response and the associated proteins have been observed in all cells, from bacteria, to fungi, to plants, to animals. What does this suggest about the evolutionary origin of the stress response?