RAMA UNIVERSITY

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FACULTY OF AGRICULTURAL SCIENCES & ALLIED INDUSTRIES

TECHNIQUES IN PLANT PROTECTION MSH-304

LECTURE 05

SDS-PAGE for Molecular Weight Determination: An Overview of the Process

To determine the molecular weight of an unknown protein, you should separate the sample on the same gel with a set of molecular weight standards. After running the standards and the unknown protein sample, the gel is processed with the desired stain and then de-stained for about 12 to 14 hours to visualize the protein bands.

After running the gel, you should then determine the relative migration distance (*Rf*) of the protein standards and the unknown protein. The migration distance can be determined using the following equation:

$$Rf = \frac{\text{migration distance of the protein}}{\text{Migration distance of the dye front}}$$

Note: You can use a ruler to measure the migration distance (in centimeters) from the top of the gel to every major band in the gel. Alternatively, an appropriate software may also be used to determine the *Rf* values of the resulting bands.

Based on the values obtained for the bands in the standard, the logarithm of the molecular weight of an SDS-denatured polypeptide and its relative migration distance (*Rf*) is plotted into a graph. Please take note that you will generate a linear plot for most proteins if your samples are fully denatured and the gel percentage is appropriate for the molecular weight range of the sample. If you get a sigmoidal curve, it means that the sieving effect of your matrix is either too large that it restricts the penetration of the molecules into the gel or is nearly negligible that it allows protein molecules to migrate almost at their free mobility.

Interpolating the value from this graph will then give you the molecular weight of the unknown protein band. Please note that the accuracy of this method in determining the molecular weight of an unknown protein typically ranges from 5% to 10%. The presence of polypeptides such as

glycol- and lipoproteins usually leads to erroneous results since they are not fully coated with SDS and thus, would not behave as expected.

So for the example pictured, the unknown protein has an Rf of 0.7084. Using the equation for the linear plot we can calculate the Log (MW). (-2.0742 x 0.7084) +2.8 = 1.3305. So the inverse log is 10^1.3305= 21.4kDa for the molecular weight of the unknown protein.

Creating Ideal Separation Conditions: Some Factors to Consider

Given its importance in determining the molecular weight of an unknown protein sample, you need to select the appropriate separation conditions when doing your experiment. To do this, you need to take the following factors into consideration:

- The standard protein and the unknown protein should be electrophoresed on the same gel under identical conditions.
- Multiple data points should be generated to make sure that the data carries a statistical significance. For best results, try using at least three gels.
- In solubilizing the proteins, the sample buffer should contain reducing agents such as dithiothreitol or B-mercaptoethanol to ensure that the disulfide bonds will be broken. As you may already know, disulfide bonds reduce the effect of secondary structure on migration.
- The sample buffer should also contain SDS. SDS binds to hydrophobic protein regions to denature secondary, tertiary and quaternary structures and give a net negative charge on the proteins. SDS causes proteins to unfold to random, rod-like chains without breaking any covalent bonds in the process. This causes proteins to lose their biological functions without damaging their primary structures.

Keep in mind that the unknown protein should be within the linear range of the standard curve to allow for the accurate determination of its molecular weight. In addition, the amount of the unknown protein should match the corresponding standard to increase the accuracy of the results.

Protein Isolation

The Bradford assay is a fast and efficient colorimetric assay to determine the protein concentration in a wide range of solutions and is widely used for protein content of cell fractions and protein concentrations for gel electrophoresis. It is often done before an SDS-PAGE analysis. Using the reagent Coomassie Brilliant Blue G-250, the solution undergoes a visible change in color when the dye binds to proteins via van de Waals and electrostatic interactions, causing a shift from a dark red to a deep blue. This is a result of stabilization moving the absorbance maximum of the dye from 470 to 595 nm. Tested samples typically take less than five minutes to reach the indicative measures of 595 nm absorbance concentration, making the Bradford assay both reliable and quick.