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Utilization of SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel) Electrophoresis in Protein Purification

Elfira Jumrah[1], Sudding[1], Alya Amalia Luqman[1], Dwiny Zachrina[1], Rifqiani Khairunnisa[1], Sri Hardiyanti[1], Virginia Dwi Gladinda[1*]

[1]Department of Chemistry, Universitas Negeri Makassar, Makassar, Indonesia

*Corresponding Address: virginiadinda18@gmail.com

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ABSTRACT

A protein can be identified based on each level of its structure. Every protein contains at least primary, secondary and tertiary structures. Only a few proteins have a quaternary structure as well. The primary structure consists of a linear chain of amino acids. One of the electric field-mediated separation methods that is often applied to analyze proteins based on their size is SDS-PAGE electrophoresis. This technique is based on the assumption that after denaturation, the polypeptide chains covered in SDS micelles have comparable surface charge densities, so that the resulting differences in electrical migration are based on their size. SDS-PAGE separates protein molecules based on their particle size and shape. This gel can be made with varying pore sizes which are determined based on the total amount of acrylamide compound added (gel concentration). The gel pore size will become smaller as the gel concentration increases so that only protein molecules that have a small molecular weight can pass through. This measure of the molecular weight of a protein is useful in mapping proteins (protein profiling). The gel staining used is bromophenol blue which functions to color proteins because it binds weakly to proteins. The higher the protein concentration of a sample being electrophoresed, the resulting band will appear clear and thick. However, if the protein concentration of a protein is low, the resulting band will appear thin.

Keywords: Electrophoresis, Protein, SDS-PAGE

I. INTRODUCTION

Protein is one of the macronutrients that humans really need, because apart from being an energy contributor, protein also has an important role in the growth and repair of cells in the body and helps maintain health conditions. Protein needs during childhood to adolescence are greater in accordance with the growth and development processes that are occurring during that period (WHO, 2004). Protein is a food substance that is very important for the body, because apart from functioning as fuel in the body, it also functions as a building and regulating substance. Proteins are polymers of amino acids linked by peptide bonds, protein molecules contain the elements C, H, O and N which fats or carbohydrates do not have (Winarno, 2008). Proteins are polypeptide structures consisting of one or more long chains of amino acid residues. They perform a wide variety of organismal functions, including DNA replication, transporting molecules, catalyzing metabolic reactions, and providing structural support to cells. A protein can be identified based on each level of its structure. Every protein contains at least primary, secondary and tertiary structures. Only a few proteins have a quaternary structure

as well. The primary structure consists of linear chains of amino acids (Sanvictores & Farci, 2022).

In medical applications such as therapy, diagnostics or other research, protein can be used but must have very high purity and not contain pyrogens. For this reason, protein purification is very important. Protein purification is a process used to remove impurities and improve the quality of the protein produced. Protein purification can be carried out using polyacrylamide gel electrophoresis which allows protein grouping based on molecular weight and charge. One method that can be used for protein purification is the SDS-PAGE method. The SDS-PAGE method is a protein separation based on differences in protein molecular weight in an electric field (Khatmizarullah, et al, 2021). The mechanism for determining the molecular weight begins with the immunoglobulin that has been obtained being inserted into the gel well at the top, where the gel is a collecting gel buffer with larger pores (Senggagau & Manja, 2020).

II. RESULTS AND DISCUSSION

a. Electrophoresis

Electrophoresis is a technique for separating a particle or species or ion or colloidal particle based on its ability to move through a conductive medium, usually a buffer solution, in response to an electric field. Technically, electrophoresis is the term given to the migration of charged particles as a result of direct electric current or DC (Direct Current). Gel electrophoresis is one of the main techniques in molecular biology. The basic principle of this technique is that DNA, RNA, or proteins can be separated by an electric field. The buffer or buffer solution used in the electrophoresis process functions to maintain pH balance and provide ions for conductivity during the electrophoresis process. In this case, the molecules are separated based on their rate of movement by electromotive forces in the gel matrix. The transfer rate depends on the molecular size, agarose gel concentration, DNA fragment size, voltage, concentration and pH of the buffer used, as well as the concentration and ability of intercalate dyes such as EtBr (ethidium bromide) used in staining (Siswoyo, 2019).

b. SDS- PAGE (Sodium Dodecyl Sulfate-Gel Polyacrylamida)

One of the electric field-mediated separation methods that is often applied to analyze proteins based on their size is SDS-PAGE electrophoresis. This technique is based on the assumption that after denaturation, the polypeptide chains covered in SDS micelles have comparable surface charge densities, so that the resulting differences in electrical migration are based on their size. In the reduction mode of analysis, reducing agents such as mercaptoethanol, dithiothreitol, etc., inhibit the disulfide bridges of proteins making it possible to analyze their subunits. In fact, SDS-PAGE is considered an automated instrumental version of the old model sodium dodecyl sulfate polyacrylamide plate gel electrophoresis (Hajba, et al, 2023). The use of SDS PAGE electrophoresis is that it can provide an overview or visualization of the size of the target protein, where there may be other impurity proteins present. The more protein impurities there are, the visualization shows the purification quality is less good. Meanwhile, the good quality of the purification results can be demonstrated by visualizing a single protein band that is thick and has minimal contaminants (Alami, et al, 2022). This electrophoresis uses two types of gel, namely separating gel and stacking gel. Separating gel functions to separate proteins based on molecular weight. Stacking gel functions to equalize the protein starting point before running. The use of SDS whose function is to denature proteins because SDS is a detergent which causes bonds in proteins to be broken to form proteins that can be eluted in the gel, SDS to make negatively charged proteins, mercaptoethanol functions as a base to cut disulfide

bonds in proteins so that the protein structure becomes linear (Oktaviani, et al, 2024). Proteins that are denatured with SDS will produce negatively charged proteins so that when placed in an electric field they will move to the positive pole or anode. The migration of proteins in the gel is proportional to their size so that smaller proteins will move faster than large proteins. So that proteins that move in an electric field are separated based on their molecular weight, a porous matrix is needed. The matrix used is polyacrylamide, a polymer whose monomer is acrylamide (Kembaren & Rachman, 2005).

Proteins have quaternary, tertiary, secondary, and primary structures. In electrophoresis, the primary structure is needed for protein migration on the polyacrylamide gel. Heating is needed to denature the protein so that it becomes the primary structure, but if the heating is at a temperature that is too high for a long time it will result in total denaturation resulting in damage to the protein which causes the protein to not be visualized on the polyacrylamide gel. Apart from that, by heating we can find out and determine the resistance properties of proteins to certain temperatures.

Proteins will experience changes in chemical structure due to heating or denaturation, namely breaking bonds in molecules. The development of denatured protein molecules will reveal the reactive groups in the polypeptide chain. Next, rebinding will occur to the same or adjacent reactive group. If enough bond units are formed, the protein will coagulate. If the bonds of the protein reactive groups hold all the liquid, a gel will form. However, if the liquid separates from the coagulated protein, the protein will precipitate. In the denaturation process, protein peptide bonds cannot be completely broken because the primary structure of the protein remains the same after the denaturation process occurs. Proteins can denature at temperatures of 50–80°C. The denaturation process in this protein can be caused by many factors, such as the influence of heating, pH, salt, or stirring. Each method has a different effect on protein denaturation. Applying heat can have an effect on protein (Triyono, 2010).

In general, proteins are colorless, so it is necessary to provide a dye that is able to detect the location of the protein during the electrophoresis process. With color, the protein can be seen and electrophoresis can be stopped before the protein drops out of the gel. One example of a dye that can be used, namely bromophenol blue, functions to color proteins because it can bind weakly to proteins (Silitonga, 2022). Small proteins will move faster than larger proteins. The relative molecular mass (Mr) of a protein can be measured using a standard protein (protein marker) whose Mr is known by comparing the relative mobility value (Rf). Rf protein is a comparison of the distance between the starting point to the protein band and the distance from the starting point to the end point of electrophoresis (Febrina, et al, 2022). The SDS-PAGE method has a relatively shorter testing time.

The results of the SDS-PAGE method are in the form of protein bands that precipitate, after staining the protein bands are read based on molecular weight. The flow of protein molecules in the gel will form a protein band pattern. Thick protein bands indicate the number of proteins with the same molecular weight at the same band position. Bands can form or appear due to a number of sample particles getting stuck at certain points in the gel due to the electric charge on the sample moving towards the cathode (Hames, 2004). The gel that has been stained and analyzed shows protein bands forming in each well. If the volume injected into the gel well is larger, the resulting ribbon will be thicker. This follows the principle of the movement of charged molecules, which means that the electric field allows charged molecules to move freely, and molecules with the same charge and size will collect in the same band or adjacent bands (Salsabila, 2024).

c. SDS (Sodium Dodecyl Sulfate)

SDS is an anionic detergent that can coat proteins, largely in proportion to their molecular weight, and impart a negative electrical charge to all proteins in the sample. Glycosylated proteins may not migrate, as it is expected that protein migration is based on its molecular weight and polypeptide chain mass, rather than its attached sugars. The use of SDS functions to denature proteins because SDS acts as a detergent which causes bonds in proteins to be broken to form proteins that can be eluted in gel (Oktaviani, 2019).

d. Gel Polyacrylamida

Polyacrylamide gel (PAG) is the most common matrix for protein electrophoresis. Amino, carboxylate, and imidazole groups in proteins are the main contributors to the net charge of proteins. At extreme pH, groups such as hydroxyl, guanidino and sulfhydryl can also influence the net charge. The charge contribution of this group depends on the pKa value and the pH of the surrounding medium. The net charge of a protein depends on the isoelectric point (pI) and the pH of the environment. At pH values above pI, the net charge of the protein is negative. In polyacrylamide gel electrophoresis (PAGE), charged proteins migrate in an electric field and their mobility depends on the net charge or charge-to-mass ratio. The PAG matrix also has a filtering effect on protein migration which can help their separation (Sharma, 2020).

e. Basic Principles

The basic principle of this method is protein denaturation by SDS with molecular separation based on molecular weight by electrophoresis method using polyacrylamide gel. When the protein is transferred into a gel and electrophoresed using electricity, the protein migrates through the pores of the polyacrylamide gel. Proteins will separate based on molecular size. The level of purity of a protein can also be seen from the SDS-PAGE electrophorgram in the form of protein bands. The more protein bands are formed, it shows that the protein is not pure. The concentration of extract protein affects the quality of the protein bands formed in the process of analyzing protein characteristics and the speed of protein band formation (Putri, et al, 2021).

f. Image of SDS-PAGE Results

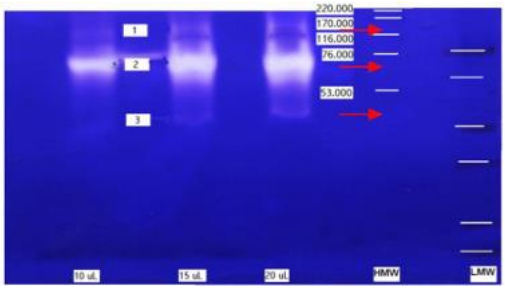

Figure 1 Zymogram results using casein substrate with sample concentrations of 10 µL, 15 µL and 20 µL. (1: band 1, 2: band 2, and 3: band 3 (Agustini, 2019)

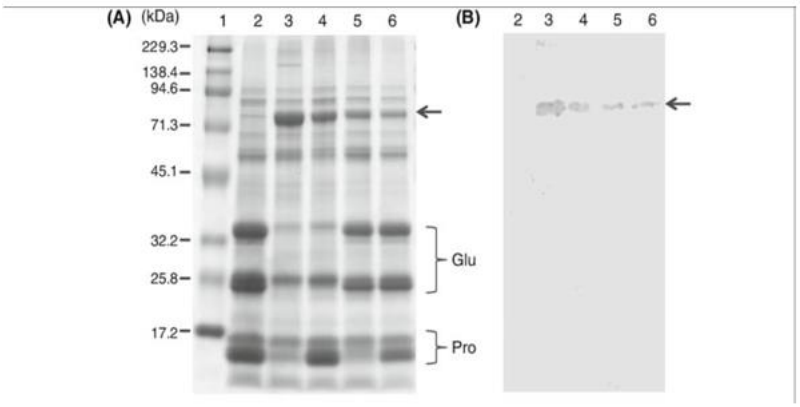

Figure 2 SDS-PAGE and western blot detection of the a0 subunit (Takaiwa, 2019)

g. Equality

The results of electrophoresis in the form of bands can be calculated by calculating the Rf (Retardation Factor) of each band using the formula:

$$Rf = \frac{\text{Distance of protein movement from starting place}}{\text{Distance of color movement from starting place}}$$

Then the Rf value is entered into the linear regression equation with the formula:

Y= a + bX

Information:

Y = molecular weight

X = sample Rf value

III. CONCLUSION

SDS electrophoresis is an analytical technique for separating proteins based on their molecular weight. When proteins are separated by electrophoresis through a gel matrix, the smaller proteins migrate more quickly due to the less resistance of the gel matrix. Other influences on the rate of migration through the gel matrix include protein structure and charge. In addition, the higher the protein concentration of a sample, the clearer the resulting band will appear. However, if the protein concentration is low, the resulting band appears thin.

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