Isolation and purification of proteins essay

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The purpose of the experiment was to isolate and recognize varying protein solubilization and assaying methods by using bovine liver protein. The experiment implicated the impact of different types of solvents like ethanol, water, PBS, PBS+1% Triton x-100, and PBS+2% SDS on protein solubilization. Bradford and Ghosh/Dumbroff methods were used to calculate the amount of the dissolved proteins in the solvent. SDS-PAGE electrophoresis was used to separate the polypeptides in the mixture and was visualized by coomassie brilliant blue and silver staining.

The techniques employed in the experiment are fundamental aspects in the field of proteomics. Introduction Amino acids are the basic building blocks of proteins that are joined by peptide bonds to form a peptide chain. These chains then conform to a special arrangement and interact with other peptide chains forming complex macromolecules known as proteins (Horton, et al. , 1996). The amino acid content of a particular protein of interest can be determined and extracted through solubilization using detergents (Carpentier, et al. , 2005). Solubilization of proteins is made possible by detergents due to the amphipathic behavior of the latter.

A detergent has a hydrophilic head and a hydrophobic tail. The head is composed of a polar carboxylate group whereas the hydrophobic tail is a long alkyl chain (Schmid, 1996). Detergents exist in water as micelles, which are spherical clusters of hundreds of carboxylate ions dispersed throughout the water phase (Schmid, 1996). The hydrophilic portion faces outwards while the lipohilic tails point towards the center of the micelle. This behavior is similar to the lipid bilayer that forms the cell membrane, giving detergents the ability to dissolve proteins (Carpentier et al. 2005). Examples of

detergents are PBS, PBS+1% Triton x-100, water, ethanol, and PBS+2% SDS which may be utilized in protein solubilization, thus facilitating identification and isolation of protein contents (Carpentier et al. , 2005). Solubilization of proteins is made possible by detergents due to the amphipathic behavior of the latter. A detergent has a hydrophilic head and a hydrophobic tail. The head is composed of a polar carboxylate group whereas the hydrophobic tail is a long alkyl chain (Schmid, 1996).

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Peptide bonds can be broken down by ionic detergents like sodium dodecyl sulfate (SDS), which has effectively been used in membrane proteins (Laemmli, 1970). SDS has the ability to denature proteins into smaller fragments, which are then subjected to polyacrylamide gel electrophoresis (PAGE), allowing separation of these fragments based on molecular weight (Cleveland, et al. , 1977). Triton x-100, an example of a non-ionic detergent, possesses either polyoxyethylene or glycosidic moieties on its hydrophilic head, thereby disrupting linkages between proteins and lipids and between lipids as well.

This property makes it easier to isolate membrane proteins (Laemmli, 1970). At low salt concentrations, similar to physiologic levels (for instance the concentration of salt in plasma), many proteins are soluble (Seddon et al. , 2004). However, as the concentration increases, the solubility of the salt in the solution is increased and the protein is salted out or precipitated. This property may vary according to the type of salt used and detergents may be required for further dissolution (Marshal, 2011).

A study by Inyang and Iduh showed an increase in protein solubility with increasing ionic strength or salt concentrations and this property was attributed to the globulins present in the molecule or the greater activity and binding capacity of the chloride ions to the positively charged protein groups (Inyang and Iduh, 1996). Phosphate buffered saline (PBS) is a widely-used solution that prevents protein denaturation by stabilizing the pH of the solution and by allowing the water molecules within the buffer to interact with the protein (Crowther, 1995).

Variations in the pH of a solution is known to alter protein conformation and therefore its adhesion or binding to the cell membrane (Jiang, et al. , 1990). Due to the charges present on protein surfaces, these molecules are surrounded by water when placed in aqueous solutions, behaving like scattered hydrated units (Oakley et al. , 2003). However, dessicated or precipitated proteins do not dissolve readily in water unless sufficient hydration is done.

Therefore, it is of particular importance to allow adequate hydration prior to solubilization (Oakley et al. 2003). Proteins also dissolve in alcohols such as

ethanol up to a certain extent depending on the character of the protein being dissolved (Oakley et al. , 2003). The observed behavior of proteins when mixed with ethanol is such that the non-polar side chains are exposed and the peptide groups are brought inside, producing a rod-like conformation with alpha-helix structures. Therefore, proteins with alpha-helices are stable in ethanol but the rest are unstable in most polar s olvents (Oakley et al. , 2003).

The term proteomics originated in 1994, with its history being closely associated with clinical chemistry (Hortin, et al. , 2006). Proteomics deals with the identification, quantification, and structural and functional analysis of proteins (Hortin et al. , 2006). There are several ways of quantifying proteins. The Bradford assay which is discussed below is one of the most commonly used methods in protein quantification (Seevaratnam et al. , 2009). The Bradford assay makes use of the Coomassie brilliant blue (CBB) G-250 (CBBG-250) dye.

The dye exists in three forms: cationic, neutral and anionic. The anionic form is able to complex readily with proteins due to hydrophobic forces and Van der Waals interactions (Compton and Jones, 1985) and appears blue with a maximum absorbance of 590-595 nm (Compton and Jones, 1985). The Bradford assay method is able to quantify proteins in micrograms through the binding of CBBG-250 to protein. Binding of the dye to the protein produces a color change from red to blue that causes an alteration in the light absorption detected through spectrophotometry from 465 nm to 595 nm (Bradford, 1979).

Increase in the intensity of the blue color signifies increase in protein concentration (Bradford, 1979). Other than the Bradford assay, the Coomassie blue dye has also been used in staining protein bands in polyacrylamide gels that were subjected to SDS-PAGE since it allows visualization of the protein bands (Patton, 2002). The disadvantage of the Coomassie dye is its binding to arginine and lysine, thereby limiting its use to specific proteins (Bradford, 1979).

Methods of improving the dye have been attempted although with limited success. The advantages of the Bradford assay are the consistency of the results and the short time required for the assay (protein binding is completed in 2 minutes). The complexes created by the dye and the protein remain stable in the solution for an hour. Substances such as cations and carbohydrates are not able to interfere with the assay (Bradford, 1979). The presence of alkaline reagents may be controlled with the use of buffers (Bradford, 1979).

However, large amounts of detergents such as SDS and Triton X-100, and commercial glassware may affect results by decreasing the intensity of the dye via dilution and refraction, respectively (Bradford, 1979). The use of cell membrane components also may affect results since it acts as a colloidal suspension (Marshal, 2011). Protein quantification using the Bradford assay is done by first diluting the protein of interest since the spectrophotometer is able to determine concentrations within the range of 2 µg/ml to 120 µg/ml (Bradford, 1976), which is analogous to the proteins with arginine residues and hydrophobic components.

An absorbance curve is plotted in parallel with the absorbance of a standard amount of a known concentration of proteins that possess arginine and hydrophobic moieties such as bovine serum albumin or BSA (Bradford, 1976). Another method of protein analysis, the Dumbroff/Ghosh method, does not make use of spectroscopy and has less interference from detergents compared to the Bradford assay (Dumbroff, 1988). The procedure makes use of the absorption of the solid phase of the protein on Whitman filter paper which is then stained with Coomassie blue.

The proteins absorbed in the filter paper appear as spots of varying colors, the intensity of which is directly proportional to the amount of protein present (Dumbroff, 1988). Similar to agarose gel electrophoresis which is used in DNA analysis, proteins may be separated based on their molecular weight and charge using polyacrylamide gel electrophoresis or PAGE. The principle behind this procedure makes use of the speed by which proteins of varying weights and charges are able to travel through a medium, which is the polyacrylamide gel.

The proteins would appear as bands in the gel after staining with Coomassie blue dye or silver stain (Pineiro et al. , 1999). SDS-PAGE is actually a modification of the standard electrophoresis in which the presence of the negatively-charged SDS overwhelms the native charge of the proteins hence separating the molecules based on mass alone (Horton, et al. , 1996). Proteins in polyacrylamide gel electrophoresis can be visualized by using silver stain. This is achieved through a reduction reaction between the silver ion (Ag-) and the protein (Horton, et al. 1996). Silver staining techniques are not affected by external factors such as temperature, solvent quality, and

developing times (Horton, et al. , 1996). Protein staining using CBB dye is widely-used due to its reproducibility and high sensitivity (Horton, et al. , 1996) even though the results may be affected by the presence of other substances and its affinity for basic amino acids alone. In general, CBB dye interacts with basic amino acids namely, lysine, arginine and histamine and the presence of more basic residues produce a more intense color.

Western blot method also makes use of gel electrophoresis and is widely used in protein identification and isolation. The proteins are subjected to electrophoresis in its native or denatured form and after separation, these are transferred to a membrane (nitrocellulose or PDVF) (Belec, et al. , 1994). Antibodies to the protein of interest are added which bind specifically, hence detecting these molecules Antibodies are proteins with binding sites specific to a particular molecule (Belec, et al. 1994). This characteristic binding may be used in isolating or identifying a specific type of protein within the nitrocellulose membrane (Belec, et al. , 1994).

Western blot analysis is used for the detection of specific proteins in sample. Antibodies are raised by the immune system against regions on the surface of proteins known as epitopes (Aroor et al. , 2010). Epitopes or antigenic determinants are often classifies as either continuous or discontinuous: a continuous or linear pitope typically comprises three or four adjacent amino acids over a short segment in the primary sequence, where as discontinuous epitopes comprise residues that are distant in primary sequence but brought together in the folded native conformation(Aroor et al. , 2010). Nitrocellulose/ PVDF filter used to transfer a protein form an SDS-PAGE gel. Once the protein moved onto the filter its arrangement is conserved to the

filter. The bond between the nitrocellulose/ PVDF filter and protein is called non-covalent linkage. Methanol is used to activate PVDF membrane (Aroor et al. , 2010).

The filter with the transferred protein is then exposed to a primary antibody. Primary antibody is used to find the location of the target protein. The secondary antibody is used in detection to show that the primary antibody has bound to its antigen. Visualization of the protein products results from a labeled secondary antibody that is specific for the primary antibody, such as anti-immunoglobulin G (anti-JgG). The secondary anti body can be radioactively labeled and the western blot exposed to film, or the secondary antibody can be tagged with a fluorescent compound that is detected and measured (Aroor et al. 2010).

Identification of proteins is aided by the use of mass spectroscopy (MS). The principle behind this method is based on the mass-to-charge ratio of charged particles (Moyers and McDonald 2006). A sample of a molecule, a peptide for instance, is bombarded using an electron beam, breaking it down into ions. Ions are separated according to their mass-to-charge ratio and are detected through MS (Irene, et al. , 2011). In summary, the steps involved in MS are sample preparation, chromatographic preparation, ionization and data analysis.

New improvements in MS have led to advances in proteomics (Moyers and McDonald 2006). The objective of the experiment was twofold. First, study the impact of different types of solvents like ethanol, water, PBS, PBS+1% Triton x-100, and PBS+2% SDS on protein solubilization. Bradford and

Ghosh/Dumbroff methods were used to calculate the amount of the dissolved proteins in the solvent. Second, SDS PAGE electrophoresis was used to separate the polypeptides in the mixture and was visualized by coomassie brilliant blue and silver staining.