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Editorial Open Access

An Introduction to Polyacrylamide Gel Electrophoresis

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Editorial Note

Polyacrylamide Gel Electrophoresis (PAGE) is a technique used in biochemistry, forensic chemistry, genetics, molecular biology, and biotechnology to separate biological macromolecules, mainly proteins or nucleic acids, based on their electrophoretic mobility. The length, conformation, and charge of a molecule influence its electrophoretic mobility. RNA samples are analysed using polyacrylamide gel electrophoresis, which is a powerful instrument. When a polyacrylamide gel is denatured after electrophoresis, the sample makeup of the RNA species is revealed.

The hydration of acrylonitrile by nitrile hydratase leads in the production of acrylamide molecules. Before adding water, the acrylamide monomer is a powder. Because acrylamide is hazardous to the nervous system, all precautions must be taken when working with it. Acrylamide is soluble in water and polymerizes when free radical initiators are added, resulting in polyacrylamide. Because pore size may be controlled, making polyacrylamide gels *via* acrylamide hydration is helpful. Smaller molecules can enter the pores and pass through the gel, whereas large molecules are stuck at the pore openings, allowing for a better examination of smaller molecules.

Molecules can be run in their native state, as with all forms of gel electrophoresis, preserving the higher-order structure of the molecules. This technique is known as native-PAGE. Alternatively, a chemical denaturant can be used to destroy the structure and transform the molecule into an unstructured molecule whose mobility is solely determined by its length (because the protein-SDS complexes all have a similar mass-to-charge ratio). SDS-PAGE is the name of the process. SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) is a technique for separating molecules based on their molecular weight differences. SDS molecules are negatively charged at the pH of gel electrophoresis and bind to proteins in a certain ratio, around one molecule of SDS for every two amino acids. As a result, the detergent ensures that all proteins have the same charge-to-mass ratio. The detergent binds to the proteins and disrupts their secondary, tertiary, and/or quaternary structure, resulting in negatively charged linear polypeptide chains. When negatively charged polypeptide chains are exposed to an electric field in PAGE, their mobility toward the anode varies. The logarithm of their molecular weight is inversely proportional to their mobility, or the distance traversed by molecules. The relative molecular weight of proteins can be calculated by comparing the distance travelled by each protein to the length of the gel, where the length of the gel is determined by the distance travelled by a tiny molecule such as a tracking dye.

Urea is the most often used denaturant for nucleic acids. SDS (Sodium Dodecyl Sulphate) is an anionic detergent used to coat proteins in order to transfer two negative charges (from each SDS molecule) to each of the two amino acids in the denatured protein. 2-Mercaptoethanol can also be utilised to break down disulphide connections between protein complexes, allowing the protein to denature even more. The binding of SDS to polypeptide chains in most proteins results in an equitable distribution of charge per unit mass, resulting in electrophoresis fractionation by approximate size. Because of the increased fluctuation in the ratio of bound SDS, proteins with a higher hydrophobic content such as many membrane proteins and those that interact with surfactants in their natural environment are inherently more difficult to treat appropriately using this approach. In terms of procedure, both Native and SDS-PAGE can be used to purify and separate the protein's constituent subunits. The oligomeric state is preserved in native-PAGE, which results in a band on the gel that represents the degree of activity. SDS-PAGE denatures and separates the oligomeric form into its monomers, resulting in bands with molecular weights that are indicative of the oligomeric form. These bands can be utilised to identify and evaluate the protein's purity.