

A Report on Protein Identification **Chen Ling***

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Brief Note

Proteins, also known as polypeptides, are organic compounds made up of amino acids. They're large, complex molecules that play many critical roles in the body. Proteins are made up of hundreds of thousands of smaller units that are arranged in a linear chain and folded into a globular form. There are 20 different types of amino acids that can be combined to make a protein and the sequence of amino acids determines each protein's unique 3-dimensional structure and its specific function.

Protein, like nucleic acid, occupies a unique role in vivo as the primary component of protoplasm and the material underpinning of life. Proteins play an important role in catalysing the processes of many species in vivo, regulating metabolism, resisting alien invasion, and controlling genetic information, among other things. The most significant work in biochemistry and other biology, food testing, clinical testing, disease diagnosis, biopharmaceutical separation and purification, and quality testing is protein separation and characterization.

Protein identification and analysis software performs a central role in the investigation of proteins from two-dimensional (2-D) gels and mass spectrometry. For protein identification, the user matches certain empirically acquired information against a protein database to define a protein as already known or as novel. For protein analysis, information in protein databases can be used to predict certain properties about a protein, which can be useful for its empirical investigation.

Currently, there are many methods for protein identification such as

Protein estimate using UV absorption: This approach, which is simple, sensitive, rapid, and can be recycled after identification, calculates the amount of protein by detecting the distinctive absorption of tyrosine and tryptophan at 280 nm. The benzene ring of the tyrosine, phenylalanine, and tryptophan residues in the protein molecule has conjugated double bonds, allowing the protein to absorb UV. While the absorption peak is at 280nm, the absorbance will be proportional to the protein amount. Furthermore, the protein solution's light absorption value at 238 nm and the peptide bond content are proportionate. By comparing the light abs and the protein content, the protein content can be determined.

High pressure liquid chromatography: HPLC (high-pressure liquid

chromatography) is a type of chromatography. In recent years; high-pressure liquid chromatography has become increasingly popular for protein separation and analysis. Different types of HPLC models can be used to separate the target protein depending on its size, shape, charge, hydrophobicity, function, and other features, as well as the source and experimental needs. Scientists investigated a variety of HPLC integrated technology in order to find more convenient and accurate ways for protein identification. HPLC-MS, HPLC-CE, HPLC-ITP, and HPLC-ICP-AES are the most commonly utilised combination technologies at the moment.

Method of Electrophoresis: Electrophoresis is the separation of charged particles in an electric field by an electrode acting in the opposite direction of their charge flow. Protein electrophoresis separation is a key biochemical separation and purification process. Agarose gel electrophoresis, starch gel electrophoresis, polyacrylamide gel electrophoresis, and more types of electrophoresis are available, depending on the supportive employed! The initial step in Western Blotting is to prepare protein samples. Sample preparation is a crucial stage that necessitates obtaining as many proteins as feasible. Suitable surfactants and reducing agents destroy all non-covalently bound protein complexes and covalent bond disulfide bonds to form a solution of the respective polypeptide; try to remove nucleic acids, polysaccharides, lipids, and other interfering molecules to achieve maximum solubility and reproducibility of the protein at the appropriate salt concentration.

Protein identification by Mass spectrometry: Mass spectrometry is a significant new tool for identifying and characterising proteins. The two most common methods for ionising entire proteins are Electrospray Ionisation (ESI) and Matrix-Assisted Laser Desorption/Ionization (MALDI). Two ways to characterising proteins are utilised, depending on the capability and mass

range of available mass spectrometers: "top-down" strategy and "bottom-up" strategy. In a "top-down" protein analysis procedure, complete proteins are ionised using one of the two methods described above and then fed into a mass analyzer. In "bottom-up" proteomics, the existence of proteins is determined at the peptide level. Using tryptic digestion to get masses of individual peptides produced from the protein is a popular "bottom-up" technique of protein analysis procedure. The peptides are then put into a mass spectrometer and identified using peptide mass fingerprinting or tandem mass spectrometry. The masses are then compared to an online database, and the closest protein matches are determined using probability-based scoring systems.

Protein Detection in Gels

Various approaches, such as dyes and silver staining, can be used to detect proteins separated on a polyacrylamide gel.

Dyes: Up to 0.2 to 0.6 g of protein can be detected by Coomassie blue staining, which is quantifiable (linear) up to 15 to 20 g. It's commonly found in methanol-acetic acid solutions and discolours

in isopropanol-acetic acid solutions. It is recommended that ampholytes be removed from 2-DE gels by adding trichloroacetic (TCA) to the dye and then discolouring with acetic acid.

Staining with silver: Because of its ease of use and high sensitivity (50 to 100 times more sensitive than Coomassie blue staining), it is a viable alternative to traditional protein gel staining (as well as nucleic acids and lipopolysaccharides). This staining method is especially well suited to two-dimensional gels.

Autoradiography for the detection of radioactive proteins

Autoradiography is a radioactively labelled molecule detection technology that use photographic emulsions sensitive to radioactive particles or light created by an intermediate molecule. Silver emulsions are susceptible to particle radiation (alpha, beta) or electromagnetic radiation (gamma, light), resulting in metallic silver precipitation. In the area where radioactive proteins are detected, the emulsion will form black precipitates.