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Opinion

A Quick Explanation of the Protein Purification Processes

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INTRODUCTION

Purification of the protein of interest is a crucial step in researching individual proteins. Purification of proteins is divided into four steps: Cell lysis, protein binding to a matrix, washing, and elution are the steps in the process. Protein engineering methods are critical for designing or manufacturing proteins with particular characteristics that may be used in a variety of industrial operations. As a result, they are essential in biotechnological research. Protein purification is a set of procedures for isolating one or a few proteins from a complicated mixture, which generally consists of cells, tissues, or entire organisms. Purification of the protein of interest is critical for determining its function, structure, and interactions. Purification can separate the protein and non-protein components of a mixture, then separate the necessary protein from the rest.

The most time-consuming part of protein purification is usually separating one protein from the others. Differences in protein size, physico-chemical characteristics, binding affinity, and biological activity are commonly used in separation stages. Protein isolate is the name for the pure outcome. The optimal protein purification procedure is determined by a number of parameters, including the cell utilised to produce the recombinant protein (e.g., prokaryotic versus eukaryotic cells). Mammalian proteins that are correctly folded and functional are more likely to be produced in cultured mammalian cells with the necessary post-translational modifications [1].

Extraction

If the protein of interest isn't released into the surrounding fluid by the organism, the first stage in any purification procedure is to disrupt the cells that contain the protein. The protein must be introduced into solution by breaking the tissue or cells holding it, depending on the source. There are numerous ways to do this, including repeated freezing and thawing, sonication, high-pressure homogenization, and permeabilization using organic solvents [2].

Differential solubilisation and precipitation

Precipitation with ammonium sulphate $(NH_4)_2SO_4$ is a popular initial step in bulk protein purification for isolating proteins. Increasing quantities of ammonium sulphate are added, and the various fractions of precipitated protein are collected. Ammonium sulphate can then be eliminated via dialysis. This technique has the benefit of being able to handle enormous amounts of data at a low cost. To dissolve cell membranes and retain membrane proteins in solution during purification, a detergent like sodium dodecyl sulphate (SDS) can be employed.

Ultracentrifugation

When a vessel holding a mixture of proteins or other particulate matter, such as bacterial cells, is spun at high rates, the inertia of each particle produces a force equivalent to its mass in the direction of the particles velocity. When a sample is "spun" in a centrifuge, large, small, and dense particles move outward faster than less massive particles or particles with more "drag" in the liquid. The angular acceleration imparted to the sample, which is generally quantified in reference to the g, determines the rate of centrifugation [3].

High-performance liquid chromatography

Third, proteins can be separated using high-performance liquid chromatography or reversed-phase chromatography based on polarity/hydrophobicity. Flowing a solution containing the protein through a column filled with different materials is the basic operation in chromatography.

Size Exclusion Chromatography, Ion Exchange Chromatography, Affinity Chromatography, Immunoaffinity Chromatography, and High Performance Liquid Chromatography are only a few of the chromatographic techniques available.

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