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Mass Spectrometry & Purification Techniques

Perspective

Brief Note on Protein Purification Techniques

Tefera Ejeta*

Department of Chemistry, University of Sheffield, Dhaka, Bangladesh

DESCRIPTION

Numerous protein purification processes are used in both biological and biomedical studies. The workflow for the expression and purification of recombinant proteins depends on many variables. These variables include the physical properties and biological functions of the protein, as well as whether a bacterial or eukaryotic cell line should be used to express the protein of interest. Significant advances have been made in the field of recombinant protein expression and purification methodologies, along with numerous commercially available systems and kits. However, proteins are complex macromolecules, and optimal protein expression and purification strategies must be determined empirically.

Protein purification

The best protein purification protocol depends not only on the protein to be purified, but also on many other factors, including: B. Cells used to express recombinant proteins (e.g., prokaryotic vs. eukaryotic cells). Due to its ease of use, rapid cell proliferation, and low culture costs, *E. coli* remains the first choice for many researchers for the production of recombinant proteins. Although proteins expressed in *E. coli* can be purified to relatively high levels, these proteins, especially eukaryotic proteins, may not exhibit adequate protein activity or folding. Cultured mammalian cells may provide a better way to produce properly folded and functional mammalian proteins with appropriate post-translational modifications. However, the low expression levels of recombinant proteins in cultured mammalian cells pose challenges to their purification. Therefore, highly selective and efficient capture of these proteins from crude cell lysates is required to achieve satisfactory yield and purity. To simplify purification, affinity purification tags can be fused to the recombinant protein of interest. Common fusion tags are polypeptides, small proteins, or enzymes that are attached to the N-terminus or C-terminus of a recombinant protein. The biochemical properties of the various tags affect the stability, solubility, and expression of the protein to which they are attached. Expression vectors containing fusion tags facilitate the purification of recombinant proteins.

Isolation of protein complexes

The main goal of proteomics is to elucidate the organisation of complex networks involved in protein function and important cellular processes. Analysis of protein interactions can provide valuable insights into the cellular signalling cascade involved in these processes, and analysis of protein-nucleic acid interactions is biological, including mRNA regulation, chromosomal remodeling, and transcription. It often reveals important information about the process. For example, transcription factors play an important role in regulating transcription by binding to specific recognition sites on chromosomes (often gene promoters) and interacting with other proteins in the nucleus. This regulation is required for cell viability, differentiation, and growth. Analysis of protein interactions often requires a simple method for immobilising a protein on a solid surface in the correct direction without disturbing the structure or function of the protein. This immobilisation must not compromise binding capability and can be achieved by using affinity tags. Immobilization of proteins on chips is a common approach for analysing protein-DNA and protein interactions and identifying components of protein complexes. Functional protein microarrays typically contain full-length functional proteins or protein domains attached to solid surfaces. Fluorescently labelled DNA is used to probe an array and identify proteins that bind to a particular probe. Protein microarrays provide a method for high-throughput identification of protein-DNA interactions. Immobilized proteins can also be used in protein pull-down assays to isolate protein binding partners' *in vivo* (mammalian cells) or *in vitro*. Other downstream applications, such as mass spectrometry, do not require protein immobilisation to identify individual components of protein partners or protein complexes.

Correspondence to: Tefera Ejeta, Department of Chemistry, University of Sheffield, Dhaka, Bangladesh, E-mail: jeta#@57aferet.com

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