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The Use of Biophysical Proteomic Techniques

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Abstract

The application of proteomic methods to the study of diseases is growing at an ever-increasing rate, and it has begun to fill in significant knowledge gaps regarding the etiology of disease and the development of efficient methods for the early detection and treatment of diseases. Mass spectrometry and protein separation methods like two-dimensional gel electrophoresis and liquid chromatography are examples of biophysical techniques that are an essential component of the advanced proteomic methods that are currently in use. Determining altered protein expression not only at the whole-cell or tissue levels, but also in subcellular structures, protein complexes, and biological fluids is one way that biophysical proteomic methods can be used to study disease. Additionally, these methods are being used to discover novel disease biomarkers, investigate disease pathogenesis, develop novel diagnostic methods, and identify novel therapeutic targets. Through more efficient methods for assessing a drug's therapeutic effects and toxicity, proteomic methods also have the potential to accelerate drug development. In order for biophysical proteomic methods to be more widely accepted and have a greater impact, this article discusses how they can be used to identify cardiovascular disease and other diseases, as well as their limitations and potential future research directions [1, 2].

Keywords: Proteomics; Liquid chromatography; Mass spectrometry; Gel electrophoresis; Diseases; Biophysical techniques

Introduction

Biophysical techniques have led to some of medicine's most significant discoveries [3]. The ability to observe bacteria was made possible by advances in microscopy, and the discovery of X-rays led to significant advancements in medical practice. To investigate the structure, properties, and biological functions of proteins, numerous novel methods have been developed in recent years [4, 5]. By putting these methods to use, researchers can keep an eye on how specific biological functions are made possible by the structure and dynamics of proteins. In turn, researchers have been able to use the development of modern biophysical techniques in the study of diseases. Proteomic approaches are the most promising of the various biophysical methods for disease research. In 1997, the term "proteomics" was first proposed as an analogy for genomics. The study of proteins on a large scale is known as proteomics. Protein characterization methods, like mass spectrometry (MS), and protein separation methods, like two-dimensional gel electrophoresis (DE) and liquid chromatography (LC), are the primary focus of biophysical proteomic techniques. MS has seen the greatest development in biophysical proteomic methods over the past ten years. Mass spectrometers are utilized for routine and research purposes in both industry and academia. MS has been shown to be useful in numerous other clinical and non-clinical applications, despite their primary use in academia for the analysis of proteins and peptides. Hemoglobin analysis, drug testing, and new-born screening are the most common clinical applications for mass spectrometers. The analysis of water quality and oil composition is two examples of non-clinical applications for mass spectrometers [6]. The purpose of this review is to provide a description of standard biophysical proteomic methods, to talk about recent technological advancements used in proteomic studies, and to demonstrate how proteomic methods can be used to understand complex disease states.

Discussion

Biophysical proteomic techniques

Proper protein separation for subsequent analysis is a crucial aspect of proteomics, and LC is currently the preferred method for sample preparation and Pre-MS Analysis involving protein separation. For protein separation, LC is a type of chromatography in which a liquid acts as the mobile phase and a solid or liquid on a solid support acts as the stationary phase. The most prevalent type of liquid chromatography utilized in proteomic research is high-performance LC. Compounds can be separated into less complex fractions using HPLC, which frequently enables the identification, quantification, and purification of the mixture's individual components [7, 8]. A pump that supplies the pressure necessary to move the mobile phase and analyte through the packed column, a stationary phase that separates the analytes and a detector of the separated analyte are the most crucial components of a HPLC system. Analyte detection is influenced by the mobile phase's flow rate, the solvent(s) used, and the strength of the analyte's interactions with the stationary phase. Even though LC hasn't made as many advances as MS has, some of its most recent ones are making it more popular. Capillary electrochromatography and ultra-high-pressure LC are two examples, both of which improve the sensitivity and resolution of proteins detected in subsequent steps while also speeding up the separation process. By locating proteins with extreme biophysical properties or low concentrations, other pre-fractionation methods like differential centrifugation affinity chromatography and free flow electrophoresis can also be used to improve the dynamic depth of proteome analysis.

The severity of an infarction is correlated with the amount of certain serum biomarkers, like troponin I and troponin T, in clinical practice. Due to a variety of factors, including the cost and lack of sensitive immunoassays for distinct proteins, testing and validation of novel proteins has produced few new candidates for clinical studies. However, new validation methods based on MS are emerging, and

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they may have the potential to generate biomarkers for the early or sensitive detection of cardiovascular diseases. Candidate biomarkers of extensively fractionated samples were identified by LC-MS/MS and then verified by quantification with multiple reaction monitoring (MRM)-MS and immunoassays in two recent studies aimed at identifying protein biomarkers for cardiovascular disease. More than 100 potentially novel differentially expressed proteins were found after a planned myocardial infarction (PMI) in one of these studies, which collected blood from patients prior to and during the PMI. Some of the proteins that are expressed differently are known clinical markers that are currently used to diagnose cardiovascular disease (CVD). Using accurate inclusion mass screening (AIMS), differentially expressed tissue proteins found in a discovery proteomic analysis were analytically quantified in peripheral blood in another study. For sequence confirmation of a potential biomarker, AIMS monitors masses previously identified in tissue using an LC-MS/MS method like Orbitrap-MS and then incorporates these proteins into a software inclusion list. Positive candidate identification necessitates a precise mass and charge state. The capability of AIMS to identify false positive candidates and/or markers that are present below the detection limit is one of its advantages. High-throughput identification and the ability to concentrate on a select group of potential candidates are made possible by this resolution. Consequently, AIMS is at least four times more effective than undirected LC-MS/MS experiments at identifying peptides of interest. MRM-MS experiments are set up with the AIMS-generated peptide precursors and fragment ions. MRM-MS or immunoassay analysis of the differentially expressed proteins in the peripheral plasma of patients with PMI or spontaneous MI and controls suggests that the potential biomarkers are exclusive to MI. In order to identify a subset of high-confidence markers that may be of clinical significance, this study employed three distinct MS methods [9, 10].

Conclusion

The way we study diseases is changing as a result of the proliferation of proteomic technologies and their significant advancements. Mass spectrometers are getting smaller and cheaper all the time, and they will eventually be used as a standard analytical tool in clinical applications in a manner similar to immunoassays. The analysis of clinically relevant samples has been expanded by biophysical proteomic methods, which have the potential to revolutionize the way diseases will be diagnosed and treated in the future. When compared to the clinical methods that are currently in use, the application of LC-MS assays can sometimes provide greater sensitivity and specificity while necessitating smaller sample volumes—something that is crucial when collecting samples from pediatric patients. With improved MS machines, software, and separation methods, protein quantification by LC-MS/MS will continue to advance. The current work on improving multiplexed assays using LC-MS/MS will reduce the cost of individual assays, and the capability to automate LC-MS/MS runs will be especially useful for clinical laboratories. However, utilizing proteomic technologies remains fraught with numerous difficulties. It is still extremely difficult to fully qualitatively or quantitatively characterize any proteome. The enormous variety of post-translational modifications and single nucleotide polymorphisms add complexity to proteins. The best mass spectrometers are put to the test by the large number of low-abundance proteins that make up the dynamic range of proteins in samples. The inability of many proteomic studies' statistical designs to accommodate the intricate multivariate analysis remains a limitation. Researchers, businesses, regulatory bodies, and clinical labs will need to work closely together if the numerous potential biomarkers discovered by proteomic techniques are to be successfully translated into routine clinical use.

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