Research Techniques Made Simple: Mass Spectrometry for Analysis of Proteins in Dermatological Research

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Identifying previously unknown proteins or detecting the presence of known proteins in research samples is critical to many experiments conducted in life sciences, including dermatology. Sensitive protein detection can help elucidate new intervention targets and mechanisms of disease, such as in autoimmune blistering skin diseases, atopic eczema, or other conditions. Historically, peptides from highly purified single proteins were sequenced, with many limitations, by stepwise degradation from the N-terminus to the C-terminus with subsequent identification by UV absorbance spectroscopy of the released amino acids (i.e., Edman degradation). Recently, however, the availability of comprehensive protein databases from different species (derived from high-throughput next-generation sequencing of those organisms' genomes) and sophisticated bioinformatics analysis tools have facilitated the development and use of mass spectrometry for identification and global analysis of proteins, summarized as mass spectrometry-based proteomics. Mass spectrometry is an analytical technique measuring the mass (*m*)-to-charge (*z*) ratio of ionized biological molecules such as peptides. Proteins can be identified by correlating peptide-derived experimental mass spectrometry spectra with theoretical spectra predicted from protein databases. Here we briefly describe how this technique works, how it can be used for identification of proteins, and how this knowledge can be applied in elucidating human biology and disease.

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Description: This article, designed for dermatologists, residents, fellows, and related healthcare providers, seeks to reduce the growing divide between dermatology clinical practice and the basic science/current research methodologies on which many diagnostic and therapeutic advances are built.

Objectives: At the conclusion of this activity, learners should be better able to:

- Recognize the newest techniques in biomedical research.
- Describe how these techniques can be utilized and their limitations.
- Describe the potential impact of these techniques.

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Abbreviations: Ab, antibody; H-CDR3, heavy-chain complementarity determining region 3; LC, liquid chromatography; LC-MS/MS, liquid chromatography tandem mass spectrometry; MS, mass spectrometry

SUMMARY POINTS

What mass spectrometry for analysis of proteins does:

- Enables direct analysis of protein amino acid sequences, allowing for identification of unknown proteins (e.g., new autoantigens in disease)
- Enables analysis of changes in global protein expression, for example, in epidermis or other organs under different experimental conditions

LIMITATIONS

- Limits in the detection of proteins in very complex samples, requiring reduction in complexity of samples of interest (e.g., by affinity purification)
- Nondetection of a protein of interest in complex samples does not exclude presence of the protein, and detection of a peptide characteristic for one protein may not be specific for this protein because peptides can be shared between proteins (i.e., protein interference problem
- Experienced bioinformaticians are needed to interpret the complex MS results

INTRODUCTION

Basic dermatological research that uses genetic and cellular techniques has resulted in significant advances, allowing for precise diagnosis and optimized therapy of skin disease, as illustrated for autoimmune blistering diseases (Kasperkiewicz et al., 2017). Only recently has a more global proteomic picture in dermatologic (and other) conditions emerged, allowing new insights of clinical relevance. For example, for pemphigus vulgaris, it was shown how various monoclonal anti-desmoglein 3 autoantibodies contribute to the polyclonal serum response and how the amount of each monoclonal antibody (Ab) changes over the course of disease (Chen et al., 2017). In another study, proteomics was used to identify differentially expressed proteins relevant to filaggrin-deficient atopic eczema (Elias et al., 2017), potentially yielding new therapeutic targets. Additionally, previously unknown interaction partners of autoantibodies in dermatologic and other autoimmune conditions were successfully identified by proteomics (Miske et al., 2016; Schepens et al., 2010).

In this review, we focus on use of liquid chromatography tandem mass spectrometry (LC-MS/MS) for protein identification because it is currently the most practical means of direct and global protein identification (Domon and Aebersold, 2006).

MS-based proteomics consists of the following stages, which will be briefly described: (i) isolation of the protein

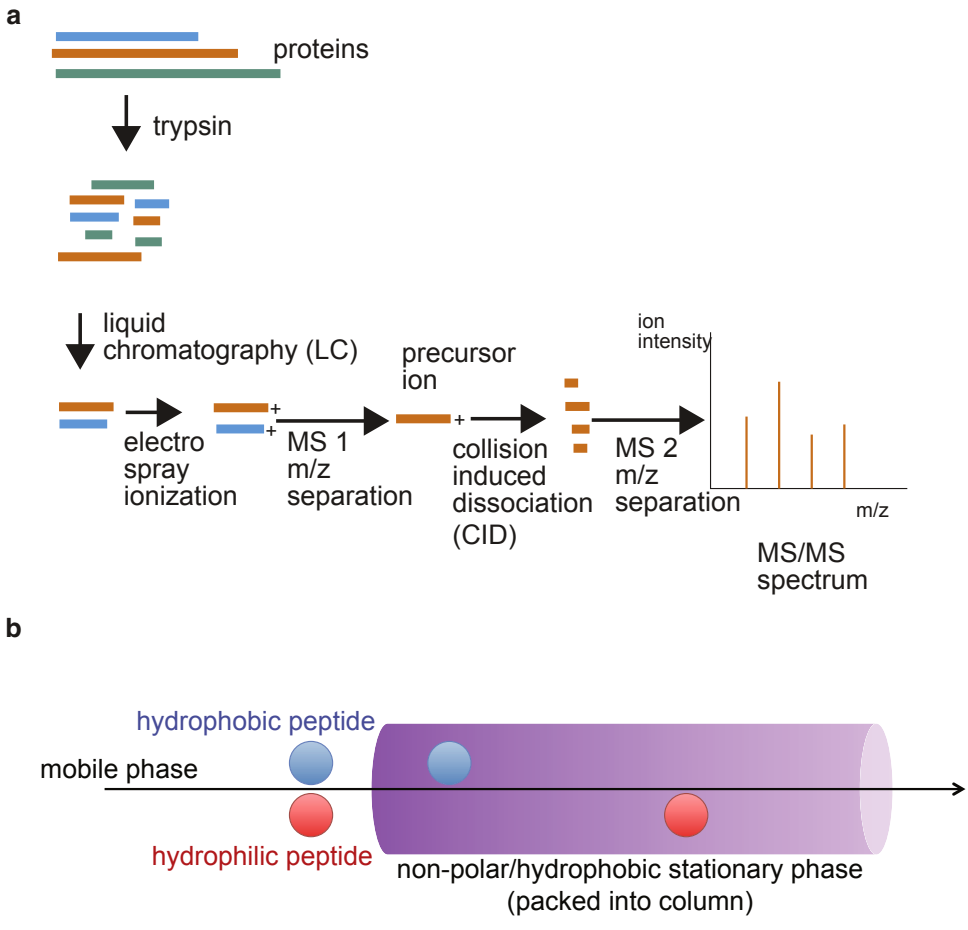

Figure 1. General steps of a typical LC-MS/MS experiment. (**a**) After isolation during the experiment of interest, proteins are treated with proteolytic enzymes (e.g., trypsin), then subjected to liquid chromatography (explained in **b**). Separated peptides are then ionized (i.e., by exposing drops of peptide-containing eluate from LC to a strong electric field, an atomic gas is formed) and separated by their mass (*m*)-to-charge (*z*) ratios in the first mass spectrometer (MS1). Precursor ions of a given *m/z* are then further fragmented by CID, and the ion fragments are separated again (MS2). Resulting fragment ion spectra are recorded and analyzed as detailed in the text. (**b**) The basic principle of reverse-phase LC. The most hydrophobic peptides interact best with the nonpolar stationary phase, whereas the least hydrophobic components elute first. Complete elution off the column, including the most nonpolar peptides, is ensured by gradually increasing the concentration of nonpolar solvents in the mobile phase. CID, collision-induced dissociation; LC, liquid chromatography; LC-MS/MS, liquid chromatography tandem mass spectrometry; *m*, mass; MS, mass spectrometry; MS/MS, tandem mass spectrometry; *z*, charge.