



Editorial

The Proteomic Revolution in Laboratory Medicine

Mass spectrometry has a rich history, starting with the discovery of the electron and the construction of the first mass spectrometer by J.J. Thompson (Nobel Prize in Physics, 1906). Since that time, numerous improvements in the instrumentation were introduced and many other Nobel Prizes were awarded, including the latest one in 2002 (Chemistry) to John B. Fenn and Koichi Tanaka for developing electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) techniques. Clinical Chemists have used mass spectrometry, in combination with gas chromatography, and later with liquid chromatography, to measure a myriad of small molecules including drugs, metabolites, vitamins, pesticides, and trace metals. Biological mass spectrometry, which has the capability to analyze proteins and nucleic acids, is a relatively new technique which rapidly progressed after the development of the soft ionization techniques (such as ESI and MALDI). In parallel to the development of new ionization techniques, we also witnessed an amazing revolution in new mass analyzers, in addition to the traditional ones (such as the Quadrupole), including ion-traps, time-of-flight, Fourier transform ion-cyclotron resonance, and Orbitrap. The combination of various mass analyzers to develop hybrid machines resulted in unparalleled mass resolution, mass accuracy and mass range, leading to very reliable identification of analytes with high sensitivity and throughput. These developments were also complemented by new bioinformatic algorithms, sample preparation techniques, development of novel reagents for quantitative analysis and other advances. The combined developments altogether, now allow identification and quantification of thousands of proteins in very complex mixtures, something that is also known as “proteomics”. Despite the fact that mass spectrometry is not the only available proteomic technique, it is certainly the most powerful and most highly utilized technology. For a historical overview and current status, see Ref. [1].

We can state with confidence that proteomics and mass spectrometry have revolutionized the following experiments over the last 20 years: reliable protein identification, resolution of complex proteomes in a matter of days, multiplexing quantitative assays for many proteins/peptides at the same time, unparalleled specificity without the need for binding reagents (such as those used in ELISAs), and relative ease in identifying complex post-translational modifications of proteins, nucleic acids and other biomolecules. Could we speculate that mass spectrometry is rapidly becoming the method of choice for just about every analytical problem? Certainly, mass spectrometry has been used, as mentioned earlier, in many areas of Clinical Chemistry as a specialty technology over many years, but it is now expanding to areas including routine analysis of steroid hormones (replacing the less specific immunoassays) and other analytes such as Vitamin D and immunosuppressants for therapeutic drug monitoring. Many believe that over the next 10 years or so, mass

spectrometry may have the capability to replace many ELISA-based immunoassays and be able to quantify any protein in complex biological mixtures without the need for binding reagents. Examples in the literature point to the fact that mass spectrometry may have unique advantages in measuring simultaneously not a single immunoreactive species, but multiple fragmented species of the same protein (e.g. parathyroid hormone, intact molecules and its fragments; this has been demonstrated already) [2], active and inactive hormones and other peptides and proteins, etc. However, we need to also realize that mass spectrometry, as it stands now, is not without limitations.

One of the most important limitations of mass spectrometry is in the identification and quantification of individual proteins in complex biological mixtures without an enrichment step. We all know that ELISAs can achieve this very easily with sensitivities down to approximately 1 pg/mL [3]. Since quantitative mass spectrometry of proteins relies on the identification of one or more peptide(s) in a sea of millions of other peptides, generated by trypsin digestion, there is tremendous competition for ionization between the peptide of interest and other peptides, even after an initial chromatographic step. This leads to suppression of full ionization and a tremendous loss of sensitivity, since the peptide of interest is ionized to a much lesser degree than when present in pure form. Additionally, the various pre-analytical steps of current protocols introduce significant variation in the quantification of these peptides, thus compromising precision. Head-to-head comparisons between ELISAs and quantitative mass spectrometric techniques for proteins currently show that ELISA is at least three orders of magnitude more sensitive and an order of magnitude more precise than the current selected-reaction monitoring (SRM) assays based on mass spectrometry [4]. Despite the fact that there are now databases which allow easy development of mass spectrometric techniques for any protein (e.g. the SRM Atlas Database; www.srmatlas.org), the limitations mentioned above still exist [5]. Many investigators have proposed ways of prior enrichment of the peptides of interest, for better and more sensitive quantification [6], but these techniques compromise throughput. It is thus our opinion that it will still take some time and more improvements in instrumentation, software and high-throughput sample pre-treatment, before mass spectrometry will pose a major threat to replacing current immunoassay methodologies. Having said that, and as stated earlier, the already-mentioned significant advantages of mass spectrometry qualify this methodology as a premier discovery technique, at present [7–9].

Most of the work that has been done already on proteomic analysis with mass spectrometry deals with the use of this technique for biomarker identification, for studying protein-protein interactions, pathway analysis, and post-translational modifications. For Clinical

Chemists, the application of mass spectrometry for biomarker discovery is of particular interest [7–9]. In this respect, mass spectrometry can easily resolve, as mentioned earlier, complete proteomes of cell lines, fluids, tissues and other biological material, making it an ideal technique for developing large catalogues of proteins in such specimens. Among these proteins, the next step in biomarker identification usually includes a strategy for selection of the best candidates by using various approaches, as we describe elsewhere [8], but this is a very difficult task since most of the applied criteria for biomarker selection are relatively arbitrary [10]. After selection of candidates, the next step in biomarker discovery, which is biomarker verification and validation, could be a very difficult step since, at this point, quantitative assays and large numbers of clinical samples are necessary. These difficulties in biomarker selection, verification and validation are major contributing factors in the realization that very few biomarkers have been discovered using mass spectrometry at present [7–9]. On the other hand, the ELISA methodology is very capable of analyzing one protein at a time, but in a high-throughput and highly sensitive fashion. In the last 5 years, we have witnessed the commercialization of a large number of ELISA assays and it is predicted that over the next 5–10 years, ELISA assays may become available for just about every known human protein. Such assays need careful validation for specificity, something that is not done rigorously at present. However, still, the ability of mass spectrometry in identifying fragments, post-translational modifications, multiparametric analysis and identification of mutated proteins will remain major advantages in our quest for finding novel biomarkers.

In this *Special Issue of Clinical Biochemistry*, we have collated a number of reviews from expert groups dealing with diverse subjects related to proteomics and mass spectrometry, and their applications in the clinical laboratory. We hope that this collection will be highly useful to Clinical Chemists, Technologists and other Laboratory Medicine professionals and to industry, since it provides a snapshot as to where mass spectrometry and proteomics are at the moment, and where these technologies are likely to be in 10–15 years. We thank all the authors for their contributions and the reviewers for their invaluable help with the assessment of the manuscripts.

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