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While definitely not a new technology, mass spectrometry (MS) has seen incredible growth over the past 25 years. Mass spectrometry has rapidly evolved to the forefront of analytical techniques; its ability to analyze proteins is the major driving force in the field of proteomics. MS instrumentation has increased approximately 5-fold in sensitivity every three years. The level of performance that is achievable with MS today allows scientists to study proteins in ways that were inconceivable a quarter century ago. This review of the history of MS over the past 25 years is timely in that it encompasses two of the biggest developments, electrospray and matrix-assisted laser desorption/ionization (MALDI), which have enabled many of the uses of this technology today.

INTRODUCTION

While it has the appearance of taking the analytical world by storm, mass spectrometry (MS) has existed in one form or another for over a century. Many scientists, however, remember the mass spectrometer as that big, user-unfriendly instrument in the cellar of their chemistry department. Most undergraduate scientists' familiarity with MS was limited to a single laboratory demonstration and no real hands-on experience. Very few courses taught the fundamentals of MS and most textbooks devoted little space to this technology. How times have changed! Mass spectrometry has now become an essential analytical tool in a variety of disciplines, including organic and inorganic chemistry, protein science, biochemistry, physics, and more. Most large private and academic institutions now have large programs that are centered on MS or rely heavily on this technology. Over the past quarter century, MS has moved from the basement to the penthouse.

Why did it take so many years for the power of MS to be recognized? The reasons can be traced to key technological developments that have occurred in the past 25 years. The most important development was the invention of new ionization techniques, specifically electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).

Discovery of ESI and MALDI

While ESI's early practical history began with the work of Malcolm Dole, who described it in studies of ions from synthetic polymers in 1968 (1), it wasn't until 1988 that the recognition of ESI's impact on biochemical studies was first presented. At the annual meeting of the American Society for Mass Spectrometry, John Bennet Fenn presented the ESI mass spectra of a number of proteins (2). Subsequent to this presentation, Fenn published the ESI mass spectra of monomeric and dimeric forms of BSA (3). These results showed that it was now possible to analyze high molecular weight proteins using MS.

Around the same time, laser desorption was being developed as an effective method to volatilize and ionize small molecular weight compounds, including peptides. In 1987 Koichi Tanaka described the use of glycerol polymer mixtures containing fine metallic particles as a laser-absorbing matrix for the analysis of high molecular weight (i.e., 22,000 Da) polymeric molecular ions (4). In 1988, Tanaka published results of using soft laser desorption combined with time-of-flight MS to measure the proteins lysozyme (14306 Da) and chymotrypsin (25717 Da) (4). Both Fenn and Tanaka were awarded the 2002 Nobel Prize in Chemistry for their development of soft desorption/ionization methods for MS analysis of biological macromolecules. Another key step toward the effective use of laser desorption/ionization for the analysis of proteins, and one that many have argued should also have been recognized with a Nobel Prize, was Franz Hillenkamp's use of organic matrices to obtain mass spectra of proteins with masses of greater than 10,000 Da (5). Developed over the last 25 years, these new ionization techniques, along with existing ones, have rendered just about every compound class analyzable through MS. The development of ESI and MALDI had an enormous impact on biochemical analysis and it is difficult to imagine the inception of proteomics without these two methods.

Development of New Mass Analyzers

With the ability to analyze almost every class of molecule, the rapid growth and use of mass analyzers

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quickly followed. A commercial quadrupole ion trap, the Finnigan MAT, which traps and mass-analyzes ions using a three-dimensional (3-D) quadrupolar radiofrequency electric field, was introduced in 1983. The popularity of the quadrupole ion trap is primarily due to George Stafford Jr.'s discovery of the mass-selective axial instability scan (6). This discovery converted a simple ion storage device into a quadrupole ion trap mass spectrometer that has applications across a wide variety of areas. Earlier work using quadrupole ion traps was based either on mass-selective stability for mass analysis or on its use as ion transmission or storage devices. The fundamental difference between the mass-selective axial instability mode and previous methods is that with the new method all ions created over a specific time period are captured within the ion trap and sequentially ejected onto the detector. The second major breakthrough from Stafford's group was the discovery that adding helium gas to the trap greatly improves the mass resolution and sensitivity of the instrument (6). The ion trap quickly became the primary instrument for conducting proteomics because of its ability to conduct tandem MS (MS/MS) analysis of complex mixtures of peptides, generated by enzymatic digestion of proteome samples such as cell lysates. Ion traps are almost ubiquitous in analytical laboratories worldwide and serve as both GC and LC downstream MS detectors.

Another important development in mass analyzers over the past 25 years has been the invention of "hybrid" instruments that bring together the best of two different analyzers. For example, the triple quadrupole time-of-flight (Q-TOF) was introduced in 1984 (7). This instrument had the ion selection and tandem MS capabilities of a triple quadrupole combined with the resolution of a TOF spectrometer. While this pioneering design used axial injection from the triple quadrupole into the TOF tube, several years later, two different Q-TOF instruments using orthogonal injection to improve performance were introduced (8,9). This new configuration provided higher sensitivity and resolution over axial injection instruments. Commercial versions of both of these instruments soon followed and there are now thousands of these instruments in MS labs around the world.

In 2000, Alma Burlingame described the development and application of a MALDI-TOF/TOF high-resolution tandem mass spectrometer for sequencing peptides (10). This instrument has been commercially available for approximately six years and provides highly sensitive peptide analysis and comprehensive fragmentation information, since it uses high energy collision-induced dissociation (CID) instead of relying on postsource decay, which is widely used with MALDI-TOF instruments. The coupling of true CID with TOF technology enables both MS and MS/MS ions to be measured with high resolution and mass measurement accuracy.

The next significant breakthrough in mass spectrometry instrumentation was not so much the invention of a new technology, but rather the availability of a specific instrument type to the general MS community. For many years after its inception by Camasorow and Marshall in 1973 (11), Fourier transform-ion cyclotron resonance (FT-ICR) MS remained a highly experimental instrument that was not routinely used in laboratories. However, by the year 2000, over 300 of these instruments had been installed in laboratories around the world. The past four years have seen an even greater explosion in the availability of FT-ICR technology. This explosion has been primarily due to the efforts of Thermo Electron Corporation and Bruker Daltronics in making the technology user-friendly to mainstream proteomic laboratories. The commercially available LTQ-FT from Thermo (San Jose, CA, USA) is a hybrid linear ion trap FT-ICR, while Bruker's (Billerica, MA, USA) APEX-Qe is a triple quadrupole mass analyzer interfaced with an FT-MS. Where the ability to achieve mass measurement accuracies of 1–2 ppm and resolution in excess of 10^5 was once found in a few laboratories, this type of instrumental performance is now commonplace.

Top-down Proteomics

A recent advance in how proteins are fragmented may change the way that proteomes are characterized in the future. The classical method of MS-based proteomics is conducted by proteolytically digesting the protein mixture into short peptides, then analyzing the peptide mixture by MS. In this scheme, known as "bottom-up" proteomics, the peptides are identified by CID, which involves collisions between the peptide and an inert gas such as helium, argon, or even air in some instances. The use of CID is effective for peptides, although not as effective for larger intact proteins. In 1998 a novel method that uses electrons for conducting MS/MS of intact proteins was demonstrated (12). This "top-down" method, referred to as electron capture dissociation (ECD), produced MS/MS spectra of proteins such as ubiqutin (8.6 kDa) and cytochrome c (12 kDa). Donald Hunt's group has recently built upon this concept, creating electron transfer dissociation (ETD) for top-down characterization of intact proteins (13). Since ECD and ETD cleave proteins primarily along the backbone, both methods retain any post translational modification information that survives the ionization process, making either a better technique for characterizing sites of phosphorylation and glycosylation than CID. While there is much room for further development, top-down methods will definitely play an increasing role in future characterizations of the proteome.

CONCLUSION

Many new technologies are still being integrated into MS to improve the resolution, sensitivity, and mass accuracy. The developments made in MS technology in the past quarter century are nothing less than staggering. This short treatise cannot do justice to all of the advances that have contributed to making MS one of the premier analytical methods available today. While this article focuses on instrumental developments, a large number of sample preparation and handling

techniques share in making proteomics possible. Many of these developments have revolved around creative methods of coupling chromatographic or electrophoretic separations with MS, with the purpose of characterizing a greater percentage of proteins within a proteome. Although we are still far from being able to comprehensively characterize entire proteomes, if the current trend in development continues, this goal might be achieved within the next couple of decades.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES

1. **Dole, M., L.L. Mack, R.L. Hines, R.C. Mobley, L.D. Ferguson, and M.B. Alice.** 1968. Molecular beams of macroions. J. Chem. Phys. *49*:2240-2249.
2. **Meng, C.K., M. Mann, and J.B. Fenn.** 1988. Electrospray ionization of some polypeptides and small proteins. 36th ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, p.771-772.
3. **Fenn, J.B., M. Mann, C.K. Meng, and S.F. Wong.** 1989. Electrospray ionization for mass spectrometry of large biomolecules. Science *246*:64-71.
4. **Tanaka, K., H. Waki, Y. Ido, S. Akita, Y. Yoshida, and T. Matsuo.** 1988. Protein and polymer analyses up to *m/z* 100 000 by laser ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. *2*:151-153.
5. **Karas, M., D. Bachmann, U. Bahr, and F. Hillenkamp.** 1987. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int. J. Mass Spectrom. Ion Phys. *78*:53-68.
6. **Stafford, G.C., P.E. Kelley, J.E.P. Syka, W.E. Reynolds, and J.F.J. Todd.** 1984. Recent improvements in and analytical applications of advanced ion-trap technology. Int. J. Mass Spectrom. Ion Process. *60*:85-98.
7. **Glish, G.L. and D.E. Goeringer.** 1984. A tandem quadrupole/time-of-flight instrument for mass spectrometry/mass spectrometry. Anal. Chem. *56*:2291-2295.
8. **Shevchenko, A., I. Chernushevich, W. Ens, K.G. Standing, B. Thomson, M. Wilm, and M. Mann.** 1997. Rapid "de novo" peptide sequencing by a combination of nanoelectrospray, isotopic labeling and a quadrupole/time-of-flight mass spectrometer. Rapid Commun. Mass Spectrom. *11*:1015-1024.
9. **Morris, H.R., T. Paxton, A. Dell, J. Langhorne, M. Berg, R.S. Bordoli, J. Hoyes, and R.H. Bateman.** 1996. A novel geometry mass spectrometer, the Q-TOF, for low-femtomole/attomole-range biopolymer sequencing. Rapid Commun. Mass Spectrom. *10*:889-896.
10. **Medzihradszky, K.F., J.M. Campbell, M.A. Baldwin, A.M. Falick, P. Juhasz, M.L. Vestal, and A.L. Burlingame.** 2000. The characteristics of peptide collision-induced dissociation using a high-performance MALDI-TOF/TOF tandem mass spectrometer. Anal. Chem. *72*:552-558.
11. **Comisarow, M.B. and A.G. Marshall.** 1974. Fourier transform ion cyclotron resonance spectroscopy. Chem. Phys. Lett. *25*:282-283.
12. **Zubarev, R.A., N.L. Kelleher, and F.W. McLafferty.** 1998. Electron capture dissociation of multiply charged protein cations. A nonergodic process. J. Am. Chem. Soc. *120*:3265-3266.
13. **Syka, J.E.P., J.J. Coon, M.J. Schroeder, J. Shabanowitz, and D.F. Hunt.** 2004. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc. Natl. Acad. Sci. USA *101*:9528-9533.

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