Introduction to Modern Biological Mass Spectrometry

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Editor's Note: The following is an Introduction by Professor Matsuo to the book entitled, *Biological Mass Spectrometry: Present and Future*, by Matsuo, Caprioli, Gross and Seyama. This book was the product of the Kyoto 1992 International Conference on Biological Mass Spectrometry held in September at the Kyoto International Conference Hall.

Introduction to Modern Biological Mass Spectrometry

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1 INTRODUCTION

Mass spectrometry is a technique utilizing technologies and ideas that are both 'old' and 'new,' and as a result is worthy, attractive and useful as an analytical method. Mass spectrometry was first conceived almost a century ago by Thomson, and developed into a form that would be more recognizable today through the pioneering efforts of Aston and Dempster. The application of mass spectrometry to the solution of chemical problems received wide recognition in the 1940s, and has gone through many changes to mature into the productive technique that it is today. Although the fundamental physics behind modern mass spectrometry had its basis over 100 years ago, these 'old' ideas and principles are still of direct relevance today. At the same time as employing these 'old' ideas, mass spectrometry is also a fertile ground for the development of new technologies and applications that have far-reaching effects in the physical, chemical and biological sciences. This is borne out by the ever increasing number of articles that are submitted to mass spectrometry conferences and to scientific journals. The development of 'soft ionization' techniques over the past two decades has led to mass spectrometry becoming widely used in biological science, and it is on this evolution that we would like to focus this chapter.

2 THE 'TREE OF BIOLOGICAL MASS SPECTROMETRY'

We believe that the development and influence of mass spectrometry in the biological sciences can be represented by illustrating it metaphorically as the 'Tree of Biological Mass Spectrometry,' as a tree has both its older inner core and the newer shoots that sprout from the ends of its branches (Figure 1).

Natural trees are germinated as seeds and grow by taking in water and nutrients through the roots and by producing carbohydrates via photosynthetic

Biological Mass Spectrometry: Present and Future. Edited by T. Matsuo, R. M. Caprioli, M. L. Gross and Y. Seyama

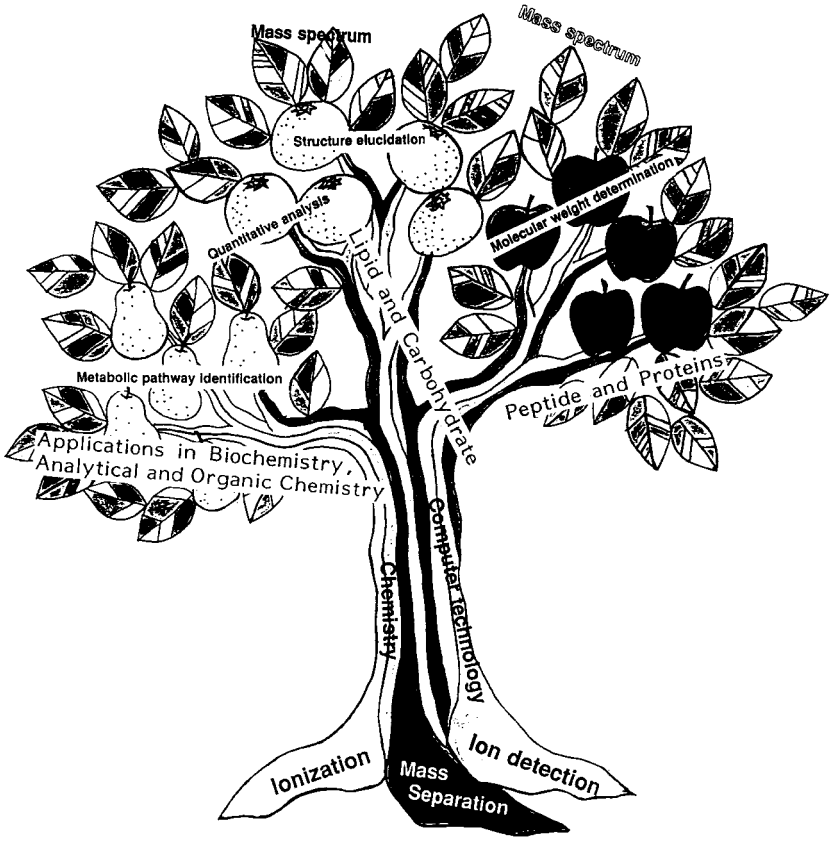

Figure 1. The 'Tree of Biological Mass Spectrometry'

processes in the leaves. At a suitable stage in the development, new branches appear and fruits are borne as the tree continues to grow. By the continued repetition of this growth and development cycle, trees can live for over 100 years. We would like to compare and contrast the evolution of biological mass spectrometry with the growth cycle we have just described.

2.1 SEED

The seed of mass spectrometry was germinated by the desire to substantiate the atomic and molecular hypotheses of the early nineteenth century. The study of electrical discharges in gases allowed scientists of Thomson's era to probe experimentally the true character of the 'corpuscles' that they believed constituted 'matter.' Although Thomson succeeded in evaluating the mass-to-charge ratio of the electron (corpuscle), his apparatus did not allow him to characterize 'ions.'

The arrival of Aston at Thomson's Cavendish laboratory led to the distinction of two isotopes of neon (m/z 20 and 22), and this work developed into the determination of precise atomic mass and isotope ratio distributions for almost all the elements. It is worth taking a moment to consider the three major developments that Aston instigated (Aston, 1933; Crowther, 1974):

1. higher intensity ion sources (current);
2. higher mass resolving power by the incorporation of velocity focusing in the mass separation device;
3. more sensitive ion detection by further development of photoplate detectors.

These three key points that allowed Aston to forge forward are factors that are still very much a consideration today.

2.2 ROOTS

Roots are fundamentally important for most trees, as they provide a basis and support upon which the tree may grow and flourish, supporting the stems, boughs, twigs, leaves, flowers and fruits. In mass spectrometry the three basic processes that occur (sample ionization, mass separation and ion detection) may be recognized as the three major roots.

2.2.1 Ionization

The ability to ionize polar, labile and involatile species has been the fundamental basis upon which biological mass spectrometry has developed. It is vitally important that we recognize the breakthroughs in ionization techniques of the past two decades. These advances have allowed biological mass spectrometry to progress and develop rapidly.

2.2.1.1 Solid sample ionization

The first breakthrough for the ionization of involatile species was made by Beckey and co-workers (Beckey, 1969) with the development of field desorption ionization. This technique depended on the use of carbon dendrite emitters. The development of the much easier to produce silicon emitters led to the ionization and detection of polystyrene species with m/z in excess of 10^4 (Matsuo *et al.*, 1979b). The detection of such heavy ions might be considered to be a milestone in high mass spectrometry.

The next ionization technique that was developed for the analysis of high molecular mass species was plasma desorption, where the fission products from the radioactive decay of californium-252 are used to desorb and ionize sample molecules supported on a solid substrate. These pioneering experiments by MacFarlane (MacFarlane, 1983) indicated one of the avenues that mass

spectrometry must take if it was to become a widely accepted analytical technique amongst biochemists.

The introduction of fast atom bombardment (FAB) ionization by Barber and co-workers (Barber *et al*., 1981) opened the door for the routine analysis of biological species by mass spectrometry. This technique evolved from the static secondary ion mass spectrometry work from Benninghoven's laboratory (Benninghoven and Sichtermann, 1978) by incorporating a liquid matrix, and was extended by the development of the higher energy (<40 keV) CS^+ ion gun by Aberth *et al*. (1982), which allowed lower base pressures in the ion source because no gas is required to generate the primary ion beam. More details of this technique are discussed by Watson in Chapter 2.1.

The use of lasers to desorb and ionize solid samples was introduced by Honig and Woolston (1963) and the FOM group (Posthumus *et al*., 1978). The recent developments using chromophobic matrices for sample support in laser desorption, introduced by Hillenkamp's group (Karas and Hillenkamp, 1988), and using ultra-fine metal introduced by the Shimadzu group (Tanaka *et al*., 1988) have led to renewed interest (a renaissance) in time-of-flight mass analysers, owing to their high sensitivity and theoretically unlimited mass range. A matrix-assisted laser desorption time-of-flight (MALD/TOF) spectrum of the haemoglobin β-chain is shown in Figure 2. There is more discussion relating to laser desorption by Hillenkamp in Chapter 2.4, and information about (tandem) time-of-flight analysers by Cotter and Cornish in Chapter 2.11.

2.2.1.2 Liquid sample ionization

Once it was possible to ionize labile, involatile samples, the objective became the direct analysis of biological samples in solution. This was especially important

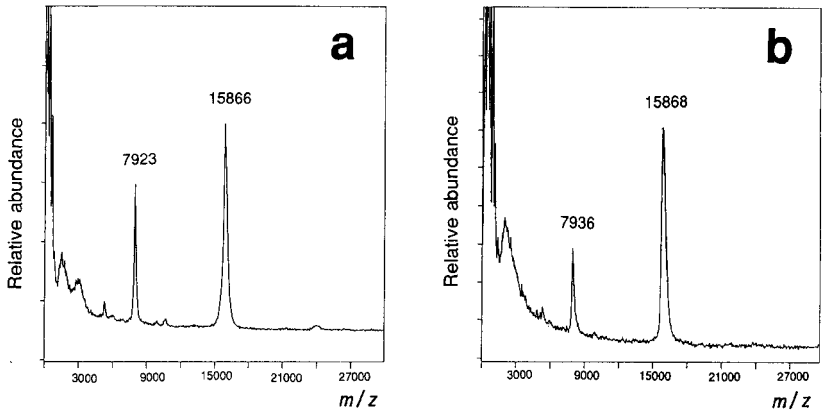

Figure 2. Matrix-assisted laser desorption mass spectra of β-globin ($C_{724}H_{1119}N_{195}O_{201}S_3$, average M.W. 15 867.2). Instrument, JEOL LDI 1700; matrix, sinapinic acid. (a) Positive-ion spectrum and (b) negative-ion spectrum

for the direct coupling of high-performance liquid chromatography to mass spectrometry. Many of the early investigations of the atmospheric pressure ionization (Horning *et al*., 1973) and hydrodynamic ion source (Simons *et al*., 1974) paved the way for the breakthrough by Fenn and co-workers (Yamashita and Fenn, 1984) and a Russian group (Aleksandrov *et al*., 1984), who prepared gas-phase ions from solution electrospray. This heralded the arrival of electrospray ionization and related methods and has led to a tremendous surge of interest in mass spectrometry by the biological community over the past 5 years. A transformed (note the spectrum has been transformed from an m/z scale to a mass scale) electrospray mass spectrum of haemoglobin β-chain is shown in Figure 3, which was produced from the similar ESI mass spectrum shown in Figure 2 in Chapter 3.7 by Wada and Matsuo. We can see that better mass resolution was achieved in the ESI spectrum than in the MALD spectrum. A detailed discussion of electrospray ionization is given by Smith *et al*. in Chapter 2.2. One of the key factors of electrospray ionization is the generation of multiply charged ions that may vastly increase the effective 'mass range' of even a modest-sized mass spectrometer, allowing the analysis of very high molecular mass compounds.

2.2.2 Mass separation

The characteristic of mass separation is the inherent capability that makes mass spectrometry such a powerful analytical tool. Besides the original sector-type instruments from early in the century, dynamic mass filters based on quadrupolar electric fields (Gaskell, Chapter 2.8), ion storage devices (Cooks and Cox, Chapter 2.9) and time-of-flight instruments (Hillenkamp, Chapter 2.4, and Cotter and Cornish, Chapter 2.11) have all been developed to make their mark in the mass analysis of biological molecules. The principal factors to be considered in mass separation for biological applications are mass range, mass resolution and mass accuracy.

2.2.2.1 Mass range

It is remarkable to note that the maximum detectable mass over the last 40 years has increased by approximately an order of magnitude per decade, as illustrated in Figure 4. In sector mass spectrometry, increasing the magnetic radius and decreasing the acceleration potential has led to the analysis of species up to m/z 3×10^5 (Ito *et al*., 1993). A small-sized ion trap has also been shown to analyse molecules of about molecular mass ca 10^5, as discussed by Cooks and Cox in Chapter 2.9. Laser desorption with TOF analysers has shown ionic species with m/z in excess of 5×10^5. These enormous increments in mass range capability have made mass spectrometry vastly more attractive to scientists whose major interests lie with large molecules. The accomplishment of high-mass analysis

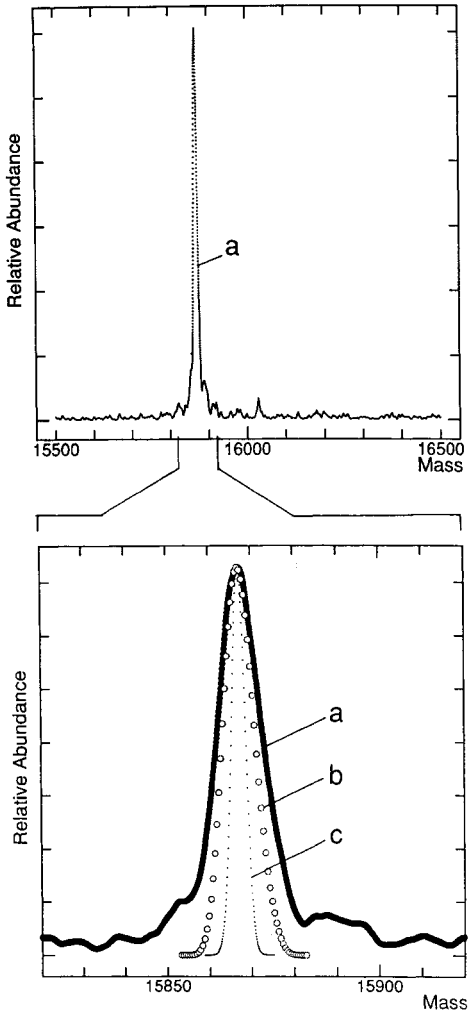

Figure 3. ESI mass spectrum of β-globin ($C_{724}H_{1119}N_{195}O_{201}S_3$, average M.W. 15 867.2). Instrument, JEOL SX102 with resolution of 2000 (10% valley definition). (a) Transformed spectrum from the 10^+ to 22^+ charged ESI mass spectrum. (b) Theoretical peak width of β-globin with a mass resolution of 2000 (10% valley definition). (c) Theoretical peak width for a single (monoisotopic) ion (e.g. CsI ion) at a mass resolution of 2000. Although the peak width became slightly wider during the transformation process, the experimental peak shape is in good agreement with theory

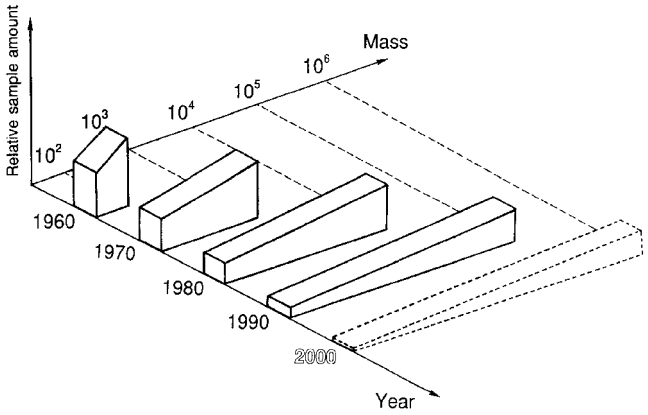

Figure 4. Extension of maximum detectable mass range at each decade. The figure also shows metaphorically that the sample amount necessary for mass measurement decreases with year and increases with molecular weight

has been stimulated to a large extent by the competing technologies of the different mass analysers.

2.2.2.2 Mass resolution

Mass resolution is another important criterion in mass spectrometry that may lead to the unambiguous identification of an unknown compound. High-resolution sector-type instruments can often supply reliable data. Recent Fourier transform ion cyclotron resonance MS has evolved to offer many significant capabilities in this area, and these are introduced by McLafferty *et al*. in Chapter 2.10. Here it must be noted that 'high resolution' and 'high accuracy' are different concepts. An apparatus that is 'accurate' can yield an order of mass accuracy that is independent of the mass resolution for a sample containing a single component. We note here that low resolving power devices can also provide sufficient mass accuracy to determine molecular masses of pure samples containing a single component. If narrow mass doublets must be analysed, however, sufficient mass resolution to separate the components is essential. The instrumental resolution is normally defined by measuring the separation of two neighbouring (isotopic) peaks for a single compound. If large biological species are being analysed without adequate resolution to separate neighbouring isotopic species, then the experimental peak width becomes broader than the instrumental resolution as a result of the isotopic distribution, as illustrated in Figure 3(b) and 3(c).

2.2.2.3 Mass accuracy

The mass accuracy of an analyser and various errors encountered in determining the molecular weight are also of great importance and are classified as shown in Table 1. Here we distinguish errors caused in determining the m/z value of ions from ambiguities that may occur during the ionization process. Systematic errors are composed of theoretical error, instrumental error and operator error. Systematic errors can be decreased by exercising care in manufacturing and using the apparatus. Precision can be evaluated by means of the standard deviation from the mean, which can be obtained after repeating a number of measurements. In the case of mass measurement, however, one cannot distinguish systematic from accidental errors, they are treated together to obtain a standard deviation. The standard deviation, of course, does not mean that the true value is located within the interval but simply specifies the degree of randomness of the measurement. Systematic errors must be checked by measuring masses of known compounds. In biological mass spectrometry, only very small amounts of sample may be available, and only a single sample loading may be possible. Even in this case, we can obtain the standard deviation by determining the molecular mass derived at every scan (ten scans, for example) and its average can give the molecular mass with standard deviation. However, if the mass spectra of ten scans were first summed and its mean calculated, no standard deviation would be obtained. Of course, both mean values are identical, but the former method is more informative.

We must also consider the ambiguity of the ionization process. The major origins might be (1) fragmentation in an ion source or in flight, (2) aggregation including polymerization, (3) structural modification such as formylation, (4) adduct attachment and (5) unexpected contamination. For most cases, we may only learn of these effects from experience by making measurements on known compounds. The questions may be solved with the help of the following techniques: (1) tandem MS, (2) different type of ionization, (3) positive- and negative-ion analysis, (4) LC/MS on-line technology and (5) different type of matrix.

Table 1. Errors in mass determination

Error in determining m/z values of ions
1.1 Systematic error
1.1.1 Theoretical error
1.1.2 Instrumental (calibration) error
1.1.3 Operator error
1.2 Accidental (statistical) error
Ambiguities occurring during ionization process
2.1 Fragmentation
2.2 Aggregation
2.3 Structural modification
2.4 Adduct attachment

2.2.2.4 Tandem mass spectrometry (MS/MS)

One of the limitations of desorption ionization in mass spectrometry has been that it did not always provide sufficient information relating to the chemical structure of the sample. Efforts to remove this limitation took the form of the development of tandem mass spectrometric methods where two analysers are used in series. In this experiment, unequivocal relationships between the sample precursor ion and the product ions are established. Early tandem experiments were performed by scanning the electric sector of a reverse-geometry instrument (Beynon *et al*., 1973). An alternative approach was linked scanning of the magnetic and electric sectors in a specific manner (Bruins and Jennings, 1978). Tandem four-sector mass spectrometry for analytical purposes was presented by McLafferty *et al*. in 1980. The use of MS/MS methodologies for biological analysis has been greatly enhanced by the work of Biemann (1992), who used mass spectrometry to obtain amino acid sequences in peptides and proteins. More detailed strategies pertaining to tandem mass spectrometric studies are given by Fenselau in Chapter 2.6. Methods of generating structurally significant product ions by ion activation are pivotal in tandem mass spectrometric studies. The details of various ion activation techniques are discussed in Chapter 2.12 by Despeyroux and Jennings (CID), in Chapter 2.13 by Morris *et al*. (SID, PD, ED) and in Chapter 2.14 by Wysocki *et al*. (SID). The neutralization–reionization phenomenon is discussed by Cordero and Wesdemiotis in Chapter 2.5.

2.2.3 Detector

The third main root of our mass spectrometric tree is the ion detection system. The main functions of the detector system are to provide (1) high detection efficiency, (2) high dynamic range, (3) small dead time and (4) positive- and negative-ion detection. The two methods most widely used are simultaneous array detection and post-acceleration of the ion beam into an electron multiplier or photomultiplier assembly. Detection systems and their applications are discussed in some detail by Burlingame in Chapter 2.7.

2.3 TRUNK

In returning to the concept of the ‘Tree of Biological Mass Spectrometry,’ the trunk carries the main communication from the roots to the branches, effectively linking the entire assembly. The core of mass spectrometry today is the electronics and control system, which is now often computer-based. The incredibly rapid advances that have been made in computer technology in the last two decades now allow us to control the majority of the instrument functions, including scanning of the mass analyser and acquiring mass spectral

data from the detector. Further, the computer allows us to process and present the data in a form that will provide the most information about the problem. The diversity of control and processing options that are now offered by most instrument manufacturers preclude the presentation of any great detail here. Organic chemistry is another important factor in the core science, and its knowledge is packed in the stem of the tree. A basic discussion on ion chemistry is given by Gross and Hu in Chapter 2.15.

2.4 LEAVES

The leaves are really the most noticeable part, or 'public face' of a tree. In addition, a number of physiological processes occur within the leaves upon which the tree depends. In mass spectrometry, the most public face of the technique is the 'mass spectra' that are generated, providing information that may be fed back into the system to provide more details about the analytical problem.

2.5 FLOWERS

The flowers of a plant also represent its 'public face' and are the site of germination for future generations. They should be as magnificent as possible in order to attract the attention of bees for efficient pollination. Mass spectrometers, ionization techniques and other analytical and separation techniques may be viewed as the pistil or stamen such that combined (hybrid) techniques such that GC/MS, LC/MS, MS/MS, FAB/MS, ESI/MS, MALD/TOF, to name but a few, can be seen as the flowers generated from suitable couplings. Although the tree/flower analogy might be stretched a bit too thin at this stage, I would still like to use such an expression, because 'flowers' are always fascinating. In any case, LC/MS, ESI/MS, MS/MS and MALD/MS are the largest flowers in biological mass spectrometry that are blooming in the 1990s, but the life of a flower is limited. MS/MS was discussed in Section 2.2.2.4, and here, LC/MS, ESI/MS and MALD/TOF are covered in some detail.

2.5.1 Liquid chromatography/mass spectrometry

Analytical methods based on the separation of mixtures according to their chemical characteristics have evolved into the fine art we know as chromatography. To combine the enormous power of the separation sciences with mass analysis, it is necessary to find suitable interfaces that will permit optimal operation of the mass spectrometer without causing any deterioration in the chromatographic performance. The first successful coupling was that of gas chromatography with mass spectrometry in the 1960s. Although this still remains a powerful combination, it is of limited use in biological sciences owing

to the high temperatures that cause thermal degradation of most biological compounds.

The next logical and biologically far more useful coupling was that of mass spectrometry with liquid chromatography, and considerable developments have taken place in the last decade. The constant limiting factor is the amount of liquid that can be introduced into the ion source of a mass spectrometer without degradation of performance and destruction of the ionization region. A reduction in the amount of eluent passing into the mass spectrometer, however, has been achieved by a variety of methods that are discussed in detail by Caprioli in Chapter 2.3. The diversity of available interfaces: moving belt (Stroh *et al*., 1985), thermospray (Blankley and Vestal, 1983), API (Kambara, 1982), continuous-flow FAB (Caprioli *et al*., 1986) and frit FAB (Ito *et al*., 1985), to name but a few, serve to reflect the importance that was placed on a facile coupling of liquid chromatography with mass spectrometry. Micro-flow LC and capillary electrophoresis have both shown promise for more efficient separation, and developments are already being made for the interfacing of these techniques.

The coupling of separation techniques with mass spectrometry should progress concurrently with developments in separation science, but it is noted that the speed of decline of any particular instrument arrangement may be just as rapid as its acceptance into the analytical community.

2.5.2 Electrospray ionization mass spectrometry (ESI/MS)

There are many discussions and references in this book relating to the relatively new technique of electrospray ionization. ESI is one of the most exciting ionization methods in the 1990s (Fenn *et al*., 1989; Smith *et al*., 1990), and has the following advantages: (1) direct ionization from solution and therefore its use as an LC/MS interface; (2) production of multiply charged ions that extends the effective mass range of the mass analyser; (3) introduction of methods to aid in desolvation of the analyte (nebulizer gas); and (4) low background from the ionization process. ESI also has some limitations: (1) dependence of ionization efficiency on both the chemical nature of the sample and on the presence of contaminants; (2) dependence of the charge distribution pattern on the inter-plate voltages in the ionization region; and (3) generation of complicated spectra when analysing mixtures (although deconvolution programs using maximum entropy algorithms may help to solve this problem). It is remarkable that we can now obtain the mass spectrum of an intact (free) protein having a molecular mass of over 10^5. It has been said that mass spectrometry technology has had two principal limitations for biochemical applications: (1) the mass range should be larger than 50 000 and (2) the amount of sample should be of the order of picomoles to femtomoles. We can now say that ESI has succeeded in removing these limitations. The technical details of ESI are discussed by Smith *et al.* in Chapter 2.2.

2.5.3 Matrix-assisted laser desorption time-of-flight mass spectrometry (MALD/TOF)

Matrix-assisted laser desorption is the other technique that has achieved high mass measurement (Hillenkamp *et al.*, 1991). The merits of MALD/TOF are (1) ultimate high mass range, (2) high sensitivity, (3) easy operation and (4) simple mass spectra. The limitations are (1) low mass resolution, (2) ambiguity of adduct attachment and (3) difficulty in combination with other analytical instruments. Details of MALD and MALD/TOF are given in Chapter 2.4 by Hillenkamp and Chapter 2.11 by Cotter and Cornish.

2.6 FRUITS

The fruits of a tree are its final product, although they may not be universally liked or appreciated. The seeds for the next generation of development are included in the fruits. The fruits generated by mass spectrometry are the solutions to problems, although the results themselves are scientific facts that are effectively independent of mass spectrometry. A few such products in terms of mass spectrometry are:

1. Molecular mass determination.
2. Structure elucidation.
3. Quantitative analysis.
4. Mixture analysis.

2.7 NEW BRANCHES

As a tree grows, branches sprout that allow the tree to expand in all directions. New branches can be generated, by grafting, for different purposes, as highlighted by the boughs carrying the different fruits in Figure 1. In biological mass spectrometry, we may view the different branches as being different areas of study and analysis, all held together by a common stem and common roots. Some of the branches of mass spectrometry are highlighted in the application chapters later in this book.

2.7.1 Peptides and proteins

To characterize a peptide or protein, it is necessary to perform the following analyses: (1) molecular mass determination of the intact species, (2) amino acid sequencing, (3) determination of disulphide bridge, (4) characterization of post-translational modification and (5) determination of ligand and intermolecular interactions.

In 1954, the amino acid sequence of insulin was determined by Sanger, and it became apparent that peptides and proteins are comprised of a limited number

of building blocks (the 20 naturally occurring amino acids) and the only substantial differences between proteins was the sequential order of these amino acids. Many methods have been developed to generate amino acid sequences efficiently, the best of which is the Edman degradation. Even today, Edman sequencing remains the quickest and most sensitive chemical method.

Peptides were first studied mass spectrometrically by Biemann in 1962 when derivatization of the peptides was necessary to make them amenable to analysis by the electron impact (EI) ionization method available at the time (Biemann, 1962). The development of field desorption (FD) ionization allowed the analysis of intact peptide molecules of molecular mass up to 1000, and became a strong weapon in the mass spectrometric armoury for analysing peptide species (e.g. Shulten, 1977; Matsuo *et al*., 1979a,b). The advent of FAB in the early 1980s opened up the analysis of peptides to all mass spectrometers that could be retrofitted with this rather simple ionization source. Peptide mapping by FD was superseded by FAB mapping, and the number of biochemists using mass spectrometric data increased greatly. Towards the end of the 1980s, four new flowers of mass spectrometry for analysing proteins reached maturity, viz. LC/MS, MS/MS, ESI/MS and TOF/MS, and it is fair to say that these are becoming invaluable to the biological community.

The state of the art of mass spectrometry in specific peptide and protein applications is discussed by Biemann in Chapter 3.1 (general strategy), by Hirayama and Akashi in Chapter 3.2 (disulphide linkages) and by Martin *et al*. in Chapter 3.3 (post-translational modifications). The relatively new approach of characterization of binding sites on receptor proteins is discussed in Chapter 3.4 by Huang *et al*. Quantification and microdialysis mass spectrometry of neuropeptides are discussed by Desiderio in Chapter 3.5 and by Andrén and Caprioli in Chapter 3.6. The study of protein molecular disease is discussed by Wada and Matsuo in Chapter 3.7.

2.7.2 Lipids and carbohydrates

Fatty acid methyl esters were the first biological materials to be analysed using mass spectrometry by Ryhage and co-workers (Ryhage and Stenhagen, 1959; Hallgren *et al*., 1959), and these spectra are almost identical with those taken using current EI mass spectrometry. This is why methyl stearate is used as a standard test compound to examine the sensitivity and mass resolution capabilities of an EI instrument.

Biologically, the most common lipids are fatty acids, a class of compounds containing a long hydrocarbon chain and a terminal carboxylate group. Biological specimens may contain several fatty acids with different alkyl chain lengths, different numbers and positions of double bonds and/or different branching characteristics. Since the physiological functions of lipids depend largely on their chemical structure, it is necessary to determine such

characteristics and to quantitate each component. As these fatty acid methyl esters were easily separated by gas chromatography, they were successfully analysed by a combination of gas chromatography and mass spectrometry (Ryhage, 1964). By using capillary chromatography, it is possible to separate more than 50 species of fatty acids by GC/MS (Yamazaki *et al.*, 1981). Other lipids, such as sterols, bile acids or triacylglycerols, are also suitable for mass spectrometry combined with gas chromatography or other chromatographic techniques. As the molecular mass of such lipids is not so high, mostly under 1000, and their fragmentation profiles yield important information about their chemical structures, EI ionization has been the mode of preference in this field. Electron impact analysis of glycerolipids is described by Kuksis *et al*. in Chapter 3.11, and Murphy and Zirrolli describe the structural analysis of lipid mediators, such as eicosanoids, in Chapter 3.10. Yano describes the analysis of microbial glycolipids, especially mycoloyl glycolipids, in Chapter 3.12. A new approach, using charge-remote fragmentation, is proposed by Gross and Hu in Chapter 2.15.

Recent instrumental developments have broadened the scope of the application of mass spectrometry in the elucidation of carbohydrates, from oligosaccharides to polysaccharides, even intact glycoconjugates. Glycosidic linkage analysis can also be determined after permethylation (Hakomori, 1964) followed by derivatization to alditol acetates (Björndal *et al*., 1967). This application also demonstrated the first successful application of mass spectrometry to carbohydrate research.

Although gas chromatography/mass spectrometry with electron impact ionization was used in the early days, and this combination is still the most widely applicable technique in linkage analysis of carbohydrates, the advent of chemical ionization resulted in a tenfold increase in sensitivity. Further, the development of FAB allowed the analysis of intact macromolecular glycoconjugates. Although the ionization efficiency of FAB-MS is poor, the technique is helpful in the analysis of biological materials without the need for complex hydrolysis or derivatization procedures. Nowadays it is possible to analyse samples in limited quantities, usually less than 1 μg of oligosaccharides, by liquid SIMS (Poulter *et al.*, 1991). Electrospray ionization has been shown to be an extremely effective and widely applicable technique in the field of glycoconjugate research and is described by Reinhold *et al.* in Chapter 3.8. In Chapter 3.9, Costello reviews the development and application of several ionization techniques to the study of glycolipids, especially glycosphingolipids.

2.7.3 Applications in biochemistry and environmental chemistry

Nucleic acids contain purine and pyrimidine bases, and all genetic information is carried in simple permutations of these units. The analysis of such species is very similar to the study of proteins, except there are fewer building blocks

to choose from. Nucleic acids are also modified, and in some cases may lead to specific dysfunctions and disease. The analysis of modified nucleosides is discussed by Crain and McCloskey in Chapter 3.13.

The elucidation of metabolic pathways can also be accomplished by mass spectrometry, and GC/MS technology has been strong in this field. The current status of xenobiotic metabolism and diagnosis of metabolic diseases is discussed by DiDonato *et al.* in Chapter 3.14 and by Millington *et al.* in Chapter 3.15, respectively.

In the 1990s, one of the major global concerns is keeping the Earth clean. Mass spectrometric techniques serve as a powerful monitoring apparatus in this regard. As one example, the presence of dioxin-like compounds in human blood is discussed by Ryan *et al.* in Chapter 3.16.

2.7.4 Applications in analytical and organic chemistry

To apply mass spectrometric techniques in the field of biology and biochemistry effectively, a knowledge of analytical and organic chemistry is necessarily important. In Part F of this book, some important methods are introduced. New separation techniques for natural product analysis are discussed by Harada *et al.* in Chapter 3.17. MS analysis is now indispensable for controlling drug abuse in major sporting events, and the methodologies used are described by Ueki in Chapter 3.18. Chiral mass spectrometry and gas-phase molecular complexes are discussed by Sawada in Chapter 3.19 and by Ohashi and Kurono in Chapter 3.20, respectively.

2.8 GROWING POINTS

We may conclude the current status of biological mass spectrometry to be as follows. It goes without saying that the development of new ionization techniques, such as ESI and MALD, have allowed great progress to be made in biological mass spectrometry. At the same time, it should be noted that the competition among the four different types of mass separation devices (sector, Q-mass filter/ion trap, time-of-flight and ion cyclotron resonance) has yielded remarkable results with regard to mass separation. It is an obvious fact, however, that a single instrument cannot satisfy all analytical demands from all different areas of study. Thus, if we are faced with characterizing a small amount of a natural sample of high molecular mass, we first try to obtain the approximate molecular mass using MALD/TOF because of high sensitivity and low contamination problems, even if the mass resolution is poor. Second, after confirming the existence of the sample, we can make efforts to correct more sample and obtain an ESI spectrum for a more precise determination of the molecular mass. Third, an enzymatically or chemically digested mixture of the

sample can be analysed to obtain more detailed information such as sequence and sites of modification using high-performance instruments.

A tree may grow in many directions; down through its roots, radially from its stem and upwards through branches and leaves. In biological mass spectrometry, the roots—ionization methods, mass separation devices and ion detectors—are firmly established, and will continue to grow slowly. The trunk of the tree will continue to grow sturdier as more advanced and reliable computer hardware and software become available and the state of the art of electronic control develops. New branches will undoubtedly continue to sprout as new areas of application are found for mass spectrometry, not only in the biological sciences but also in analytical sciences as a whole.

It is fair to say that the 'Tree of Biological Mass Spectrometry' will continue to grow and flourish year by year into the foreseeable future.

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