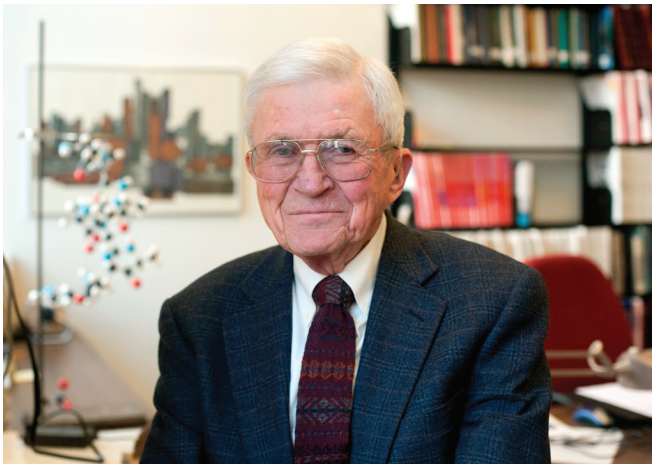

Fred McLafferty

A Century of Progress in Molecular Mass Spectrometry

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Annu. Rev. Anal. Chem. 2011. 4:1–22

First published online as a Review in Advance on February 22, 2011

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

This article's doi: 10.1146/annurev-anchem-061010-114018

1936-1327/11/0719-0001$20.00

Keywords

odd-electron ion, metastable ion, neutralization-reionization MS, top-down proteomics, electron-capture dissociation

Abstract

The first mass spectrum of a molecule was measured by J.J. Thomson in 1910. Mass spectrometry (MS) soon became crucial to the study of isotopes and atomic weights and to the development of atomic weapons for World War II. Its notable applications to molecules began with the quantitative analysis of light hydrocarbons during World War II. When I joined the Dow Chemical Company in 1950, MS was not favored by organic chemists. This situation improved only with an increased understanding of gaseous ion chemistry, which was obtained through the use of extensive reference data. Gas chromatography–MS was developed in 1956, and tandem MS was first used a decade later. In neutralization-reionization MS, an unusual, unstable species is prepared by ion-beam neutralization and characterized by reionization. Electrospray ionization of a protein mixture produces its corresponding ionized molecules. In top-down proteomics, ions from an individual component can be mass separated and subjected to collision-activated and electron-capture dissociation to provide extensive sequence information.

1. INTRODUCTION

MS: mass spectrometry

A century ago, Sir Joseph John ("J.J.") Thomson built the first instrument used to measure mass-to-charge (m/z) values of gaseous ionized atoms or groups of atoms (1–4). In the parabola mass spectra he measured in 1910, Thomson identified H_2, N_2, O_2, and CO_2—the molecules that motivated the title of this review. Further, he foresaw today's use of mass spectrometry (MS) and entitled his 1913 book *Rays of Positive Electricity and Their Application to Chemical Analysis* (4).

However, it was Thomson's mass spectrum of Ne that established the technique's unique experimental potential (5). This spectrum clearly showed the presence of isotopes with masses of 20 and 22 in an abundance ratio of 10:1 (**Figure 1**) (6, plate I); these results are consistent with Ne's atomic weight of 20.2. The subsequent determination of isotopic relative abundances extended MS applications to the exact determination of atomic weights and to the quantitative analysis of elements and isotopes (5). The latter application suddenly became important during World War II for the development of the atomic bomb—the U-235 used in the first bombs was actually separated by MS.

World War II also led to the first important applications of MS to molecules: MS quantitative analysis ensured reproducible compositions for critical refined hydrocarbons such as aviation fuel and butadiene monomer for synthetic rubber. In 1950, I joined the Dow Chemical Company, where MS was already being applied to both qualitative and quantitative analysis of molecules, mostly nonhydrocarbons. Here, I reminisce on my 60 years of this century of molecular MS.

2. DISCOVERY OF MASS SPECTROMETRY, 1910

J.J. Thomson entered Cambridge University in 1876. In 1884, at the age of 28, he succeeded Clerk Maxwell as head of Cambridge's Cavendish Laboratory; his close connection with

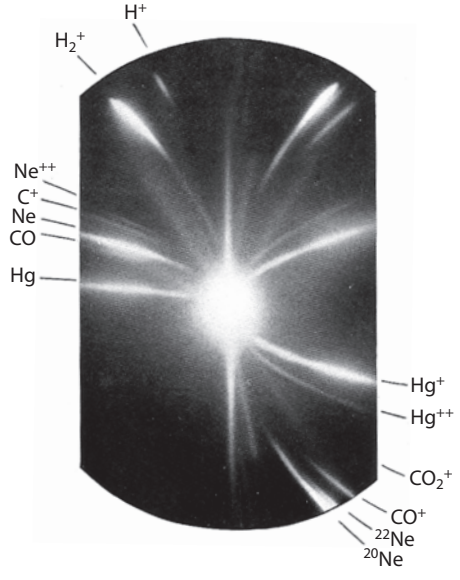

Figure 1

The mass parabolas of the Ne isotopes 20 and 22 (*lower right*) of ~10:1 relative abundance. Reprinted from Reference 6 (plate I) with permission.

Cambridge continued until his death in 1940 (1–4). His early cathode-ray experiments were crucial to the initial characterization of the electron (which he termed the corpuscle), which earned him the Nobel Prize in Physics in 1906. His 1907 book, *The Corpuscular Theory of Matter* (6), was an important advance for chemistry: It described (*a*) the role for electrons in atomic valency, (*b*) chemical bonding, (*c*) the periodic properties of the elements, and (*d*) radioactivity. Thomson had a reputation as a "clumsy experimentalist" and "never—a skillful manipulator" (3), who "wrote a clear and beautiful hand, his one manual accomplishment" (1). In 1895, he recruited for assistance with his research young Ernest Rutherford, who won the Nobel Prize in Chemistry in 1908.

When Thomson and others extended these electric and magnetic field effect studies to the beams of positive ions formed by electric discharges (cf. Goldstein's 1886 discovery of Kanalstrahlen), the experimental problems became even worse. For example, under the vacuum conditions then obtainable, fast ions could be neutralized and even reionized by background gas collisions, which affects the ions' degree of electrostatic and magnetic field deflection and thus the observed *m/z* value. For this research, Thomson recruited Francis W. Aston as his assistant (in 1922, Aston won the Nobel Prize in Chemistry). Their early work was critical to the discovery of the proton, whose low mass, relative to that of other elements, alleviated a number of experimental problems. In 1910, after making significant improvements to the apparatus, Thomson and Aston obtained (1) mass spectra of residual gas in the instrument that showed the positive ions H^+, H_2^+, C^{2+}, N^{2+}, O^{2+}, C^+, N^+, O^+, C_2^+, N_2^+/CO^+, CO_2^+, and Hg^{2+}/Hg^+. These included the first mass spectra of molecules; the electric discharge used for the ionization of the molecules also produced fragment ions from them.

3. ISOTOPES AND ATOMIC WEIGHTS, 1913–1945

Soddy proposed the name isotopes for chemically identical atoms whose radioactive properties are very different (1). Although careful atomic weight measurements had yielded whole-number values (within experimental error) of H = 1.0, C = 12.0, N = 14.0, and O = 16.0, measurements of Ne had yielded 20.2. The mass spectrum of Ne (**Figure 1**) offered the first evidence for the existence of a stable isotope, Ne-22, whose 10% relative abundance nicely corresponded to the average atomic weight, 20.2. Wide acceptance of the new concept of isotopes came with Aston's new mass spectrograph (5), which had stabilized ion-accelerating voltage and double-focusing electrostatic and magnetic deflection. This instrument demonstrated far higher sensitivity and more accurate mass and abundance values, finding Ne-21 of 0.3% abundance and the Cl-35 and -37 isotopes. Aston (5) and others extended these studies across the periodic table (Aston alone studied more than 50 elements); the high accuracy of the isotopic masses led to Aston's discovery of nuclear packing fractions.

In World War II—in which I participated as a U.S. infantryman in France and Germany—the atomic weapons program provoked a huge MS effort that included isotopic analysis for U-235 separation. The first chemical characterization of Pu-252 was performed on a nanogram sample isolated by Albert Nier on his mass spectrometer at the University of Minnesota; this separation was then repeated for bomb quantities of U-235 on the huge calutrons in Oak Ridge, Tennessee.

4. MOLECULAR MASS SPECTROMETRY, 1925–1950

For the MS of organic molecules, the early MS ionization method of electric discharge dissociated most or all of the molecular ions. Aston's 1922 book (5) describes the mass spectra of five simple molecules (of which CH_3Br is the largest) that yield vanishingly small molecular ions and many background peaks. In electron impact ionization [now termed electron ionization (EI)],

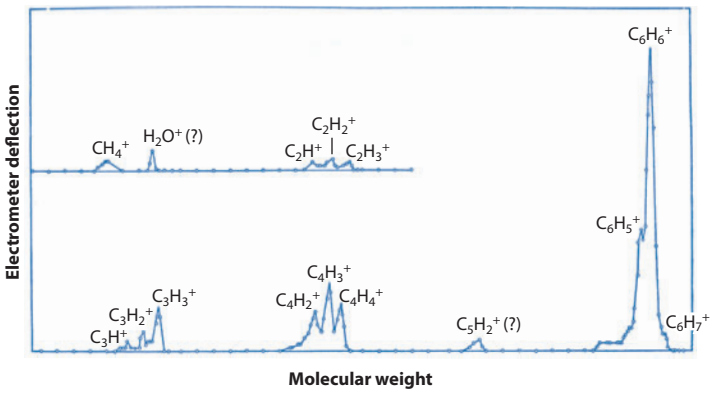

Figure 2

Electron ionization mass spectrum of benzene showing galvanometer measurements of ion currents as a function of magnetic field (7). Note the unit mass resolution between the $C_6H_6^{+\cdot}$ and $C_6H_5^+$ peaks, whose mass-to-charge values are 78 and 77, respectively. Reprinted with permission from Reference 7. Copyright 1932, American Physical Society.

a gaseous molecule is bombarded with ~70-eV electrons, ejecting an electron to form a much more stable molecular ion than that from electric discharge; such EI spectra provide far more structural information. A personal favorite example (7) from 1931, authored by Ernest Linder of Cornell University (no, he was not my student), shows the EI mass spectrum of benzene (**Figure 2**). Although its galvanometer values of ion current were measured point by point with increasing magnetic field strength, the EI mass spectrum of molecular and fragment ions closely resembles spectra measured today. Further foreshadowing today's goals in MS instrumentation, Linder's instrument "was designed especially for use with large molecules. High resolving power . . . is necessary" (7, p. 149).

This unit resolution makes the information content of an EI mass spectrum at least comparable to that of its electromagnetic spectral counterparts, which is critical to the far higher specificity required for molecular versus elemental analysis. The standard analytical techniques used then (and that I used for my master's degree in analytical chemistry), namely volumetric and gravimetric analysis, yield only a single value, so molecular specificity requires physical or chemical treatment that is unique to the analyte. Low-boiling (light) hydrocarbons were critical for the refining of fuels and monomers used in World War II, and the best available technique of fractional distillation was slow, inaccurate, and of poor sensitivity. In contrast (8), MS can yield 1% accuracy for a dozen molecular components, given that the mass spectrum of a mixture is a proportional superposition of the individual component spectra. Most spectra of light hydrocarbons contain a dozen or more mass values of exact whole-number accuracy, and their abundance values can be reproducibly measured over a dynamic range of at least three orders of magnitude [as J.J. Thomson said, "It is easy to detect a single atom. By electrifying atoms . . . they can be given as much energy as desired, and energy counts in detection" (9)]. Thus it is possible to detect 0.1% of benzene (C_6H_6) (**Figure 2**) in mixtures of the hydrocarbons C_6H_{12} and C_6H_{14}, as their EI mass spectra contain $\ll$0.1% C_6H_6 ions (*m/z* 78).

5. QUALITATIVE MOLECULAR MASS SPECTROMETRY, 1945–1960

My chemistry education at the University of Nebraska (bachelor of science, 1943; master of science, 1947), Cornell University (doctorate, 1950), and the University of Iowa (postdoctorate, 1950) did

Table 1 Proportion of presentations on molecular analysis at U.S. annual mass spectrometry (MS) meetings

Year	Number of presentations	Proportion of presentations on molecular analysis
1952[a]	23	43%[b]
1960[c]	85	48%
1965[c]	108	54%
1970[d]	181	60%
1980[d]	384	88%
1990[d]	784[e]	92%
2000[d]	1,556[e]	97%
2010[d]	3,050[e]	98%

[a]Pittsburgh Analytical Conference, MS presentations.
[b]Mainly petroleum or nonindustrial.
[c]American Society for Testing Materials E-14 Committee on Mass Spectrometry.
[d]American Society for Mass Spectrometry.
[e]Includes posters.

not include MS, as these universities, like most others, did not have expensive mass spectrometers. Luckily, when I began working at Dow, I was trained by Vic Caldecourt, who was an expert in all aspects of MS ranging from spectral interpretation to maintenance of the Westinghouse 90° sector mass spectrometer; moreover, he was building a second instrument. My real initiation into the MS scientific world took place in March 1952 at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, which was already a leader in its field. [The first U.S. MS society, the E-14 Committee of the American Society for Testing Materials (ASTM), was organized at this meeting by fewer than 50 attending mass spectrometrists; the conferences of its successor, the American Society for Mass Spectrometry (ASMS), now have more than 7,000 attendees.] The conference program listed 24 exhibitors, of which only four were for IR spectroscopy and one for Raman spectroscopy. There were two for MS: Consolidated Engineering Corporation (CEC) and General Electric Company. No company advertised nuclear magnetic resonance. At the meeting, 121 scientific papers were presented. Twenty-three were on MS; four of those were on instruments, and ten described mainly the MS analysis of molecules (**Table 1**). Only two of the latter were relevant to nonhydrocarbons of Dow interest: The first, by Friedel & Sharkey (10) of the U.S. Bureau of Mines, was on alcohols, and the second was by CEC's Sybil Rock, another pioneer in nonhydrocarbon MS, and her colleagues (8, 11). IR was then a modern spectroscopic method for qualitative molecular analysis that provided direct evidence of the presence of specific functional groups within the molecule. However, although the mass spectra of related hydrocarbons were usually different enough to allow quantitative analysis, the relationship between an MS spectrum and the molecular structure was not understood well enough for a priori identification without reference spectra. For example, although some small peaks of enhanced abundance in the spectra of eight isomers of methylpentadecane ($C_{16}H_{34}$) can be related to increased C-C cleavages at methyl substitutions, the spectra are qualitatively very similar. For all isomers, the *m/z* 43, 57, and 71 peaks (for $C_3H_7^+$, $C_4H_9^+$, and $C_5H_{11}^+$, respectively) are nearly the largest. In contrast to IR spectra, an abundant peak in a hydrocarbon mass spectrum is often a poor indicator of a structural feature.

However, the great success of MS quantitative analysis led chemical and other companies, government laboratories, and several universities to explore qualitative molecular MS. Industrial

scientists seldom published their results, which made personal acquaintances and communication especially important. Notable MS laboratories included those of John Beynon at Imperial Chemical Industries (ICI) (12, 13), Einar Stenhagen and Ragnar Ryhage at Uppsala University and the Karolinska Institute (14), Jacques Collin at the University of Liège (15), Rowland Ivor Reed at the University of Glasgow (16), Vlada Hanus at the Heyrovsky Institute of Prague (17), Lewis Friedman at Brookhaven National Laboratory (18), Frank Long at Cornell University (18), and Klaus Biemann at the Massachusetts Institute of Technology (19, 20); I apologize to the many friends and colleagues I cannot name owing to lack of space. These groups observed that the mass spectra of compounds containing heteroatoms such as O, N, S, and P—for instance, aliphatic alcohols (9)—showed more specific dissociation reactions. For example, the largest peak in the EI mass spectrum of 2-hexadecanol, -$CH(OH)CH_3$, is at *m/z* 45; it is negligible in the spectra of the methylpentadecanes. These observations led to the development of unique MS applications for molecular structure characterization, although those from industrial laboratories were often unpublished. Notable early applications resulted from the 1947 construction of a mass spectrometer by ICI physicist John Beynon, who used this instrument in a wide variety of molecular problems. His classic review article (12) and pioneering book (13) introduced the concept of high-resolution MS, in which millimass-accuracy mass measurement determines the elemental compositions of a molecule and its fragments, further defining each mass number from among many possible compositions.

6. CHEMISTRY OF GASEOUS ION DISSOCIATIONS, 1950–1964

For IR spectra, the scientific relationship between the vibrational frequency of a functional group and its molecular structure was well accepted. Although the quasi-equilibrium theory of Henry Eyring and coworkers (21) provided a fundamental understanding of simple hydrocarbon EI mass spectra, the apparent spectral insensitivity to structural change led some organic chemists to scornfully propose the so-called canvas bag mechanism, in which dissociations are induced by treatment with a hammer. How else can the $H_3CCH(CH_3)_2$ molecule that contains only CH and CH_3 groups yield an abundant CH_2CH_3 fragment? When Tal'roze & Lyubimova (22) presented their now historic evidence for the CH_5^+ ion in 1952, the ridicule became even worse ("Haven't you mass spectrometrists ever heard of tetravalent carbon?").

During this period of bad press for MS molecular structure applications in the 1950s, great progress in competing techniques such as X-ray crystallography, IR spectroscopy, and nuclear magnetic resonance was taking place. Evidence of real scientific appeal to potential MS users came from Meyerson and colleagues (23), who showed that the dominant MS dissociation of toluene gaseous ions, $C_7H_8^+$, proceeded through the intermediate formation of the cyclic tropylium ion, in which all H atoms are equivalent. Such nonclassical carbonium ions were an exciting new concept in physical organic chemistry (24). Although these authors' findings nicely explained the close similarity among, for example, the EI mass spectra of the *o*-, *m*-, and *p*-xylenes, our MS structure postulations were still challenged by the question of rearrangements.

The 1950s saw a rapid increase in careful studies on MS fragmentations of various types of molecules (see, e.g., References 10–19). The quasi-equilibrium theory defined a kinetic basis for the competitive, stepwise, unimolecular dissociations of a molecular ion formed by the expulsion of an electron from the analyte molecule (21). Ionization is easier to perform with heteroatoms such as N, O, and S; this process produces a radical site of favored intramolecular reactivity. Rearrangements, fortunately, were found to involve such specific reactions in a number of studies (25–28). Nicholson (26) even pointed out the similarity between the MS dissociation of ketone $M^{+\cdot}$ ions and that of photoexcited ketones (the Norrish type II rearrangement). In 1956 (27) and 1959

(28), I studied a collection of 4,000 reference mass spectra and found similar specific rearrangement behavior across compound classes that allowed confident predictions of structure from an unknown molecule's mass spectrum. Friends termed this behavior the McLafferty rearrangement (29–31).

GC: gas chromatography

In 1956, Dow founded its Eastern Research Laboratory near Boston and chose me to become its first director (I told friends that I could leave the MS field because it was "at its peak"). During the lab's three decades of existence, I was its director for a wonderful eight years; of its early scientists, several were elected to the U.S. National Academy of Sciences, and one (George Olah) received the Nobel Prize.

At the Eastern Research Laboratory, no mass spectrometer was available for my own research, but I did have a copy of the Dow MS reference file on IBM punch (Hollerith) cards (32). By the time I joined Dow's Spectroscopy Laboratory, its leading IR lab had amassed a world-class spectral library by acquiring small bottles of different compounds and measuring their reference spectra whenever the IR spectrometers were not in use. I copied this approach for our MS instruments (of which there were three by 1953) and ran two 8-h shifts per day. Roland Gohlke continued doing so following my departure from the Spectroscopy Laboratory in 1956; he subsequently published a collection of 2,000 Dow reference spectra (33). The collection I used from 1956 to 1964 contained ~4,000–4,500 spectra, although the ~1,500 spectra from the collection of the American Petroleum Institute consisted mostly of hydrocarbons. With this unusual data advantage for my "dry lab" research, I organized and correlated spectra of compound classes and/or structural types, which led to the publication of 15 papers between 1957 and 1963. My attempts to assign structure correlations to every spectrum led me to write a book listing the most probable structure assignments at individual mass values (34) and, ultimately, a textbook about the interpretation of mass spectra (35). Many other investigators contributed to the rapid development of applications of MS structural characterization: At the 1960 ASTM E-14 MS meeting, 85 papers were presented, of which 23 were on instrumentation, 6 on the fundamentals of ion dissociation, and 8 and 15 on the structural characterization of hydrocarbons and other molecules, respectively (**Table 1**).

7. TANDEM SEPARATION AND IDENTIFICATION METHODS, 1955–1960

A revolutionary analytical method, gas chromatography (GC), emerged in the early 1950s. I first heard about its experimental details from innovators Steve Dal Nogare of DuPont™ and H.N. Wilson of ICI at the 1954 Gordon Conference, and Roland Gohlke of my Dow MS group used their general instructions to design and assemble literally hundreds of GC/MS instruments for many Dow installations.

Passing a complex mixture through a GC column causes individual components to emerge at different times, which is ideal for quantitative analysis. For qualitative component identification, the high sensitivity of MS is favorable, but the ~1-s GC peak separation times make comparable times for MS spectral measurement desirable. Unfortunately, then-current photographic and chart recorder techniques for scanning MS spectra could not achieve this speed; a strip chart recorder pen required 1 s to go from baseline to full scale for a single peak. However, W.C. Wiley (36) at the Bendix Corporation had just developed a time-of-flight (TOF) instrument that measured mass spectra at an amazing rate of 10 kHz; the spectra could be continuously displayed on an oscilloscope. During the winter of 1955 to 1956, Bill Wiley and Dan Harrington invited Roland Gohlke and me to travel to their Southfield, Michigan, laboratory from Dow in nearby Midland to attempt to utilize our GC apparatus together with their TOF instrument, even though the latter had never been used with organic compounds. Polaroid photographs of the oscilloscope screen (**Figure 3**) reveal resolved, easily interpretable mass spectra of acetone, benzene

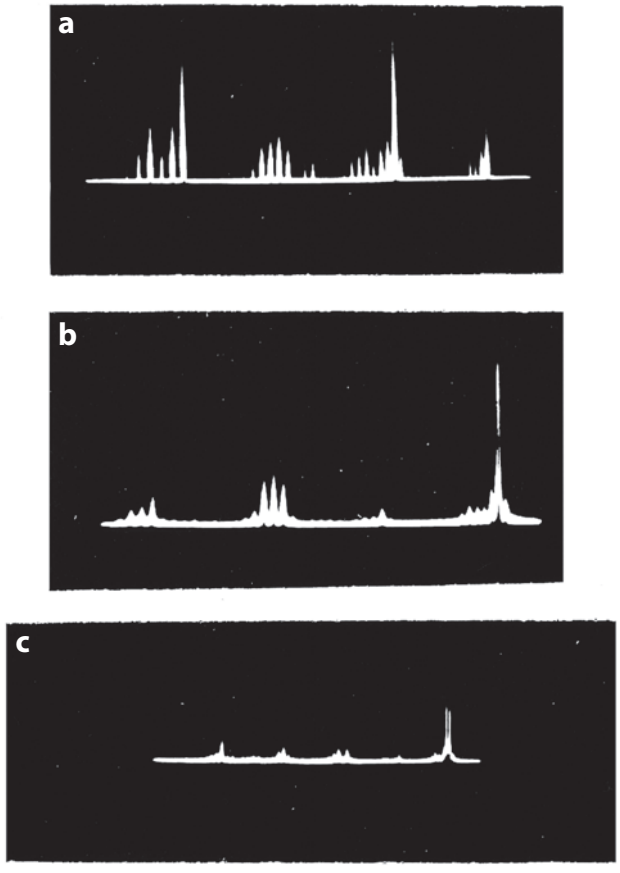

Figure 3

Time-of-flight mass spectra recorded from an oscilloscope on Polaroid film of eluted gas chromatography fractions. (*a*) Acetone (*m/z* 14–58). (*b*) Benzene (*m/z* 36–79). (*c*) Toluene (*m/z* 27–92). Reprinted with permission from Reference 38. Copyright 1993, Elsevier.

(**Figure 2**), and toluene. This first effort to interface GC with MS was only briefly or tardily described in the literature (36–38).

Interfacing the GC and MS instruments posed a significant challenge because the dominant effluent, carrier gas, could overwhelm the vacuum system. The jet separator soon solved this problem (39), but only far later did liquid chromatography combined with MS (LC/MS) become important. My claim that the previously developed Gohlke separator, results from which were never published, was the first LC/MS interface has always attracted lively interest. Around 1957, I happened to be at Dow in Midland, Michigan, attending a corporate family open house, when I was surprised to see Roland at the TOF/MS instrument, placing a finger on an open vacuum valve on the ion-source housing. Roland then dipped one finger of his other hand in CCl_4, and ~25 s later the characteristic *m/z* 117, 119, and 121 CCl_3^+ isotope peaks of CCl_4 appeared on the oscilloscope screen—but without additional peaks from the carrier fluid (blood). Gohlke himself had served as the separator. When he dipped his finger in dimethyl sulfoxide, it partitioned through him so rapidly that it shut down the MS vacuum system. At that time, Dow was urging dry cleaners,

among others, to switch from CCl_4 to Dow's less toxic chlorinated solvents, but Dow's advertisers failed to use Gohlke's compelling demonstration.

There were similar incentives to develop interfaces for LC/MS, but Baldwin & McLafferty's (40) 1973 commercial solution using chemical ionization was superseded by several others. In these solutions, electrospray ionization (ESI) (41) enhanced LC/MS just as the jet separator (39) had enhanced GC/MS.

LC: liquid chromatography

ESI: electrospray ionization

Odd-electron ion: an ion with an unpaired electron

8. DATABASES OF MASS SPECTRA: COLLECTING AND SEARCHING, 1950–2009

A serious disadvantage of GC/MS is that volatile samples are required. A great offsetting advantage, however, is that such samples have the volatility necessary to produce EI mass spectra. In contrast to most other ionization techniques, EI produces odd-electron molecular ions whose dissociations are much more structure specific, yielding spectra of far higher information content. An important early incentive to collect reference EI mass spectra was the need to understand gaseous ion chemistry, but structural assignment to the mass spectra from unknown samples became far more critical for most laboratories. By the early 1960s, the bulk of U.S. reference spectra (e.g., Reference 33) had been contributed by members of the ASTM E-14 Committee on Uncertified Reference Spectra (the American Petroleum Institute's collection had stricter rules for the acceptance of spectra). In Europe, my friend Einar Stenhagen, who had moved to Göteborg University, had gathered contributions of spectra in Europe, and together we published a collection of 6,800 different spectra in 1969. Computer sorting and checking required the expertise of my colleague Rengachari ("Babu") Venkataraghavan (44) and of Sixten Abrahamsson at Göteborg (43); Abrahamsson insisted that our publisher, Wiley, make the database available on magnetic tape. Doing so was not only a first in electronic publishing, but it became the only reasonable medium of distribution following the subsequent growth of the database. Unfortunately, during the next dozen years both Stenhagen and Abrahamsson passed away, leaving me as the sole editor. Venkataraghavan and, later, Douglas Stauffer (45) made the computer data-handling programs far more sophisticated. The 2009 ninth edition (46) contains 660,000 different spectra of 592,000 compounds; more than 600,000 spectra have visual structures, and more than 460,000 have Molfile computer structures. This database, combined with that of the National Institute of Standards and Technology (2008; 220,000 spectra of 193,000 compounds), contains 797,000 spectra of 667,000 compounds.

Two general approaches are used for the structural identification of unknown compound(s) that yield an unknown mass spectrum (47). Matching against the database should be used first, but its suggested identification(s) can and should always be checked manually (a step termed interpretation). For the first approach, I recommend (not surprisingly) the probability-based matching system (PBM) developed by my research group (48, 49) because it is clearly superior for general (e.g., GC/MS) unknowns (50). PBM calculates the statistical reliability of its predictions by weighting the structural importance of its matching to mass, abundance, and other data. PBM is also a reverse search system that matches the peaks of the reference against the unknown, not vice versa. This approach is especially valuable for mixtures; unknowns representing 85% of a mixture were identified by PBM with 48% accuracy, whereas a matching system without the reverse search function gave 27% accuracy (50). Although various specialty databases of specific compound classes (e.g., drugs) are available, the largest database yields the highest probability of a correct match (51).

If the correct match is not in the reference file, the MS scientist must follow the second, interpretation approach by using basic data, such as isotopic masses, abundances, statistics (34),

and mechanisms (35, 52). To aid this process, we developed an artificial intelligence program to supply substructural possibilities (47, 53). This self-training interpretive and retrieval system (STIRS) matches data from the unknown spectrum in 26 structurally specific classes (e.g., low-mass ion series, neutral losses) against data of the corresponding class for each reference spectrum. For the best matches of a data class, the high occurrence of a substructural feature indicates its presence in the unknown spectrum, but the chemist must examine the best matches of each class to identify possible features. In 1981, 600 common structural features were identified by STIRS (54) by use of a small list of reference spectra with computer-readable structures and (slow) computers. A STIRS rebirth should be possible, given the availability of more than 460,000 reference spectra with Molfile structures (46), as well as modern computers.

9. GENERAL ACCEPTANCE OF MASS SPECTROMETRY, 1960–1970

When I moved from Dow to Purdue University in 1964, research in and applications of qualitative molecular MS had grown substantially. At the 1965 ASTM E-14 MS Conference, 108 papers were presented, of which 28 were on instrumentation, 12 on fundamentals of ion dissociation, and 2 and 24 on structural characterization of hydrocarbon and other molecules, respectively (**Table 1**). Certain books of this period were highly useful and influential (12, 20, 30, 35, 55, 56). Carl Djerassi started a prolific MS research program (57) soon after he moved to Stanford University in 1959. In 1964 and 1967, he and his postdoctorates Herbert Budzikiewicz (later of the University of Cologne) and Dudley Williams (later of Cambridge University) published comprehensive, widely used descriptions of the mass spectral behavior of organic compounds (30, 52, 56). Chemists working with natural products rapidly became aware of MS capabilities for alkaloids (20, 56), steroids, terpenoids, sugars (30), insect pheromones (58), and sequencing of larger peptides (59). The basic theoretical understanding of the quasi-equilibrium theory (21) was extended to larger molecules (60), and mechanisms of mass spectral dissociations (30, 31, 35, 52, 56) became more easily applicable through concepts such as (*a*) localized charge and radical sites, (*b*) odd- and even-electron ions (31, 35, 52), and (*c*) distonic ions (61, 62).

Particularly importantly for several of these fields was that a mass spectrum could require less than 1 μg of sample (58). Commercial instruments had become much more user-friendly. Especially effective early efforts in computer instrument control and data acquisition and reduction foresaw the great importance of a dedicated computer for MS instrumentation. For example, Venkataraghavan et al. (63) used a DEC PDP-8 (4,000 12-bit words of core memory; 32,000 words of disk memory) with a double-focusing MS instrument; approximately 2 min following completion of the magnet scan, the printer produced a list of peak masses, abundances, and elemental compositions.

The late 1960s also saw great progress in the general acceptance of MS as a useful method with greatly improved tools for various molecular problems. Many young people joined the MS field and became crucial to its current success. At the 1970 ASMS MS Conference (the successor to the ASTM E-14 Conference), 181 papers were presented, of which 109 were on molecular MS (**Table 1**).

In 1968, I moved to Cornell University, which remains my home. The move to Cornell also expanded new areas of my own research interests.

10. TANDEM MASS SPECTROMETRY

Both GC/MS and LC/MS are systems that combine two analytical processes in which a substance isolated by the first process is characterized by the second. In 1947, Hipple (64) pointed out

that diffuse peaks found at nonintegral masses in the spectra of magnetic sector instruments can arise from metastable ions that dissociate during travel through the field-free region and before entering the magnet. Acceleration of the precursor ion as mass m_1 and magnetic deflection of the product ion of mass m_2 produce a signal at mass $m^* = m_2^2/m_1$, defining both m_1 and m_2 of the dissociation pathway. As reviewed in 1960 (13, pp. 251–62 and 279–82), such metastable peaks and their counterparts, which arise from collision-induced dissociation (CID), provide a great deal of fundamental information about gaseous ion reactions. For example, the kinetic energy released in an ion's dissociation is reflected in the metastable peak's width and shape. Note that a specific value of m^* yields multiple values of m_1 and m_2 from the above equation; improved instrumentation solved this problem by allowing separate mass analyses before (MS-I) and after (MS-II) the metastable or collisional ion dissociation.

Metastable ions: ions that dissociate during passage through the mass spectrometer

CAD: collision-activated dissociation

In 1966, T.W. Shannon and I (65) reported that such metastable dissociations could also be used to characterize the isomeric structure of an ion. The *m/z* 45 isomers $CH_3OCH_2^+$ and $CH_3CH(OH)^+$ produced very different relative metastable yields of H_3O^+ and HCO^+ ions that clearly established the presence of the corresponding isomeric partial structure in the parent molecules. Thus an MS-I dissociation of $CH_3OCH_2X^{+\cdot}$ formed and separated an *m/z* 45 product, and its MS-II spectrum matched that of the reference spectrum of $CH_3OCH_2^+$. In a similar study, T.A. Bryce and I (66) extended this tandem MS (MS/MS) technique to isomeric molecular ions; we distinguished five isomers of $C_6H_{14}^{+\cdot}$, two of $C_6H_{12}^{+\cdot}$, and two of $C_8H_{18}^{+\cdot}$, although the spectra of *o*-, *m*-, and *p*-xylene were very similar, as were their EI spectra. However, the internal energy of ions undergoing metastable dissociation can vary substantially (67, 68), which can lead to corresponding abundance changes in the MS-II spectra. Differences between the spectra of $CH_3C(OH) = CH_2^{+\cdot}$ fragment ions formed by one or by two rearrangements led us to postulate (69), incorrectly (70), that these two pathways led to ions of different structures. Compared with reactions in EI mass spectra, metastable ion dissociations occur within a narrow range of threshold internal energies, which represent reactions of lowest-frequency factors, typically rearrangements (71). Consistent with this observation, collisional activation of the ions under specific conditions in the field-free drift region raises the ions' average energies by a specific amount, far above threshold, to yield reproducible spectra (71–73). [At that time, CI was a common abbreviation for chemical ionization; currently, the terms CID and collision-activated dissociation (CAD, my preference) are used interchangeably to designate the technique.] Subsequent studies established the fundamentals of the technique (74) and illustrated its broad applications (75). It became possible to characterize the full structure of a larger molecule through CAD identification of its individual fragment ions (76).

As pioneered by the Cooks group (77, 78), CAD also became known for its utility in quantitative analysis of targeted molecules in complex mixtures (77–80). If the mass of the sought analyte's molecular ions is also produced by other mixture components, the CAD spectrum of that ion can still show the unique signature of the analyte and thereby provide selective quantitative analysis. For such applications, instrumentation is critical; the electrostatic analyzer often used as the MS-I or MS-II mass separator yields less-than-unit mass resolution. The quadrupole has a far greater resolving power, and Yost & Enke (81) showed that the triple quadrupole, first constructed at LaTrobe University (82), is an excellent solution to this problem. Q1 serves as MS-I; Q2 performs CAD; and Q3 serves as MS-II, enabling a wide variety of computer-controlled experiments (78, 81). This instrument is still widely used.

Our early MS/MS studies (83, 84) also showed that individual molecular ions from the mass spectrum of a peptide mixture can be isolated by MS-I and dissociated by MS-II and that the resulting spectrum provides extensive sequence information. If we were to extend MS/MS to polypeptides, proteins, and other large molecules, resolving powers far greater than 1,000 would

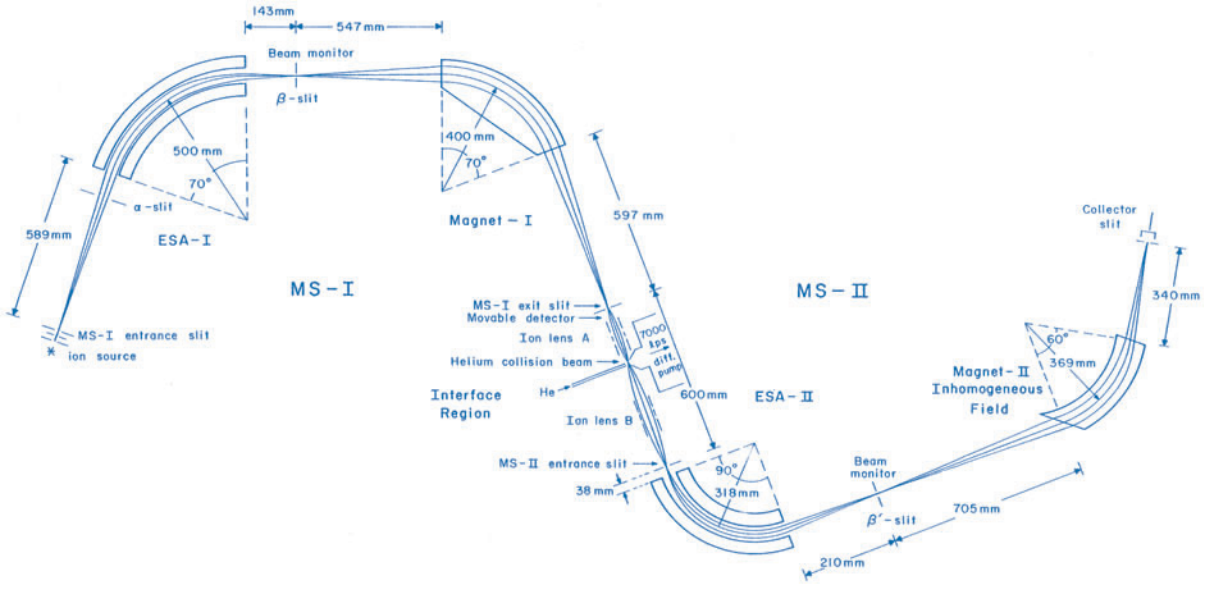

Figure 4

A four-sector EBEB mass spectrometer (85), constructed between 1977 and 1980, that was used for high-resolution tandem mass spectrometry (MS/MS) and MS/MS/MS (EB/E/B) of larger molecules and for high-resolution neutralization-reionization MS (EB/neutralization/CAD/ionization/E/CAD/B). Abbreviations: B, magnetic analyzer; CAD, collision-activated dissociation; E, electrostatic analyzer. Reprinted with permission from Reference 85. Copyright 1980, American Chemical Society.

be needed. Peter Todd, Don McGilvery, Mike Baldwin, and I (85) constructed the first tandem double-focusing mass spectrometer (**Figure 4**) for such studies. Its high-resolution MS/MS applications were valuable for diverse studies (86–88), and this so-called four-sector became the premier commercial MS instrument: Well over 100 units were sold over the next decade. However, we were able to perform MS/MS only on a heptadecapeptide, MW 2094, with ionization by fast atom bombardment (86). Proteomics had to wait for suitable ionization methods to be developed.

11. NEUTRALIZATION-REIONIZATION MASS SPECTROMETRY

One of the first experiments (85) to use this new instrument was inspired by the research of my Cornell colleague Dick Porter, who used MS to prepare unusual neutral species of selected internal energies by passing a beam of the corresponding ions, in this case NH_4^+, through a neutralizing agent of selected ionization energy (89). To perform further MS characterization of the neutrals in the instrument shown in **Figure 4**, my group (85) induced CAD of accelerated acetone cations following MS-I, electrostatic deflection of all ion products, passage of residual neutrals such as $CH_3^{\cdot}$ and $CH_3CO^{\cdot}$ through a collision chamber for ionization, and measurement of the MS-II mass spectra of products such as CH_3^+ and CH_3CO^+. This study fostered our new research area, neutralization-reionization MS (NRMS) (90). Independently, John Holmes and colleagues (91) commenced a vigorous related research program. Our four-sector instrument was critical to our NRMS research, with modifications (92) for four collision regions, in which (*a*) high-resolution MS-I-selected ions can be passed through a metal vapor to produce fast neutrals of selected internal energy; (*b*) such neutrals can be collisionally activated or (*c*) reionized and

Neutralization-reionization mass spectrometry (NRMS): a technique in which an ion beam is neutralized and the resulting neutrals are characterized by reionization and mass analysis

measured in the electrostatic analyzer as MS-II; or (*d*) the CAD spectrum of an MS-II-selected ion can be measured by MS-III (magnet-II).

As summarized elsewhere (93), NRMS has broad applicability to ion structure and analysis; the lower isomerization tendency of neutrals, versus that of the corresponding ions, is particularly useful for hydrocarbon ion characterization. NRMS is unique in that it uses stable ions to prepare unusual, unstable neutral species and to characterize their unimolecular chemistry. The species studied include carbenes; ylides; diradicals; clusters; organometallics; and hypervalent, sterically strained, and antiaromatic molecules [e.g., cyclobutadiene (96)]. Exciting NRMS research grew rapidly; the laboratories of Holmes (94) and Helmut Schwarz (95) were particularly prolific. My former postdoctorates Chrys Wesdemiotis and Frantisek Turecek created productive NRMS research groups of their own at the Universities of Akron and Washington, respectively. The four-sector instrument was shut down after the publication of our thirty-ninth NRMS paper (97).

Top-down proteomics: a technique in which a protein mixture is ionized and component molecular ions are mass separated, dissociated, and mass analyzed

12. TOP-DOWN PROTEOMICS

Yielding to the constant "persuasion" of my friends Alan Marshall and former Dow Eastern colleague Mel Comisarow, inventors of the Fourier transform (FT) ion cyclotron resonance mass spectrometer (98), I installed an FTMS instrument at Cornell in 1984 in the hope that its performance would be superior to that of our four-sector for the MS/MS of proteins. For the four-component mixture gramicidin D, laser desorption and MS/MS sequenced 12 of the 15 amino acids of the mass-1,920 $(M + K)^+$ ions from 1 pmol of one component (99). Although FTMS had an impressive mass limit of greater than 16,000 Da (100), among other promising attributes (101), the highest-mass ion recorded using radioactive Cf-252 plasma desorption was a mass-2,016 $(M + K)^+$ ion of alamethicin (102). Similar results were obtained from cesium ion desorption/ionization (103).

The transforming development in MS, certainly worthy of the Nobel Prize awarded to its discoverer, was ESI (41). John Fenn and his coworkers Craig Whitehouse and Matthias Mann were very helpful in teaching my students Kent Henry, Evan Williams, and Bing Wang how to build an ESI apparatus. These students drove this instrument from Cornell to the University of Virginia, where Don Hunt, Jeff Shabanowitz, and their colleagues (104) had already added a quadrupole ion-introduction system to FTMS. Our joint efforts led to the successful ESI introduction of protein ions into FTMS in 1989 (105).

The field of MS proteomics soon expanded dramatically in many fine laboratories around the world (106) and dominated MS meetings (**Table 1**). The number of MS proteomics publications over the past 20 years exceeds that of molecular MS publications during the previous 80 years. However, the exciting story of proteomics is too broad to treat properly here. I take the editorial liberty of citing a cogent recent review (107); below, I describe how some of our own work evolved in keeping with the 100-year perspective I attempt in this review.

The great majority of MS proteomics effort, and success, has been in protein identification at the genome level. This process involves initial digestion of a protein mixture to peptides, performance of MS/MS to identify their sequences, and matching of these sequences to DNA-predicted proteins. We term this process the bottom-up approach (108) to distinguish it from the 100-year molecular MS approach of beginning with the intact molecule, now termed top-down MS proteomics (108). Initial cleavage of the protein into peptides destroys the protein's overall sequence information and obscures posttranslational modifications; top-down, not bottom-up, proteomics allows the possibility of obtaining the full transcriptome protein structure, complete with positioning of posttranslational modifications in individual proteins. For example, 42 posttranslationally modified forms of the human HeLa cell histone H4 protein were characterized and measured

quantitatively over a dynamic range of more than four orders of magnitude, and their variation was tracked over three cell cycles (109). In many ways, FTMS—although expensive—has been the ideal technique to combine with ESI for top-down proteomics: It is currently capable of 10^6 (formerly 10^5) resolving power, 100-ppb (formerly parts-per-million) mass accuracy, subattomole sensitivity (110, 111), and high MS/MS capabilities (107).

Electron-capture dissociation (ECD): a technique in which external electrons are added to gaseous multiply charged cations to cause their energetic dissociation

13. ELECTRON-CAPTURE DISSOCIATION

CAD of peptides can provide extensive, even full, sequence information, but the amount of information obtained decreases with the size of the peptides. Thus, the bottom-up approach may yield better protein sequence coverage than the top-down technique. To improve the extent of top-down cleavages, many methods of adding energy to break the bonds between protein amino acids have been attempted; these include IR (112) and 193-nm (113) laser excitation and surface collisions (114). However, in each of these approaches, the excitation energy is randomized over the whole ion before such ergodic dissociations take place, so that most sequence-informative cleavages are at the weakest bonds. Further, the protein molecular ions, mainly $(M + nH)^{n+}$, are even electron; a well-known advantage of old-fashioned EI mass spectra is that their odd-electron molecular ions, $M^{+\cdot}$, undergo much more specific cleavages (35, 52). This alternative method for dissociating other backbone bonds was suggested for protein MS/MS (115); the serendipitous achievement of electron-capture dissociation (ECD) (116) produced these $M^{+\cdot}$ ions by very basic chemistry: $M^+ + e^- \rightarrow M^{+\cdot}$. Even more serendipitously, the recombination energy (~5 eV) released in this reaction is greater than the dissociation energies of the protein ion backbone bonds; nonergodic cleavage apparently takes place more or less randomly prior to energy randomization. Combined ECD/CAD sequence coverage is far greater: 183 of 258 bonds were cleaved in a single spectrum of 29-kDa carbonic anhydrase (**Figure 5**) (117), and 252 of 258 bonds were cleaved in 25 of its ECD spectra (118). Labile side chains such as sugar (119) and phosphate (120) are retained far better with ECD than with CAD, and ECD can occur without appreciable dissociation of noncovalent bonds. Hunt and coworkers (121) extended this method to electrostatic-focusing MS instruments such as quadrupoles and linear ion traps by electron-transfer dissociation, which has made the technique useful for bottom-up proteomics.

14. CONFORMATIONS OF GASEOUS PROTEIN IONS

The three-dimensional structures of proteins—particularly the unusually specific biochemical transformations achieved by highly specific, noncovalently bonded stereochemistry—have been intensively studied for many years. The conformational effects of ESI on a protein by its ionization and transfer into the gas phase are important for various ESI experiments; it is assumed that little change takes place when ESI is used in screening for protein-ligand agents (122) and in delineating the structures of megadalton protein complexes (123). Such conformational effects have been studied through various methods, such as H/D exchange (124), ion mobility (125), ECD (126), and IR photodissociation spectroscopy (127).

From many such studies, Kathrin Breuker of the University of Innsbruck and I (128) assembled evidence that the new gaseous environment of a simple protein following ESI can cause dramatic structural alterations. The temporal evolution of the native protein structure during and after transfer into the gas phase can involve basic side-chain collapse (which occurs on the order of picoseconds), unfolding of hydrophobic and electrostatic bonding (milliseconds), and electrostatic bond refolding (seconds to minutes), leading to the formation of new, nonnative structures (**Figure 6**). Both CAD and ECD effect minimal dissociation with proteins as large as ~75 kDa, which is apparently due to their refolding (**Figure 6**); collisional activation of larger ions just after

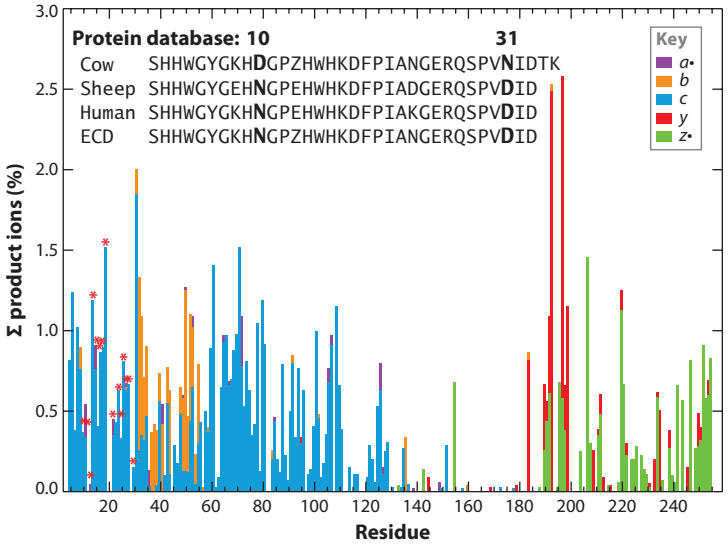

Figure 5

Backbone cleavages indicated by fragment ions in a single plasma electron-capture dissociation (ECD) spectrum (117) of bovine carbonic anhydrase (259 residues; molecular weight: calculated, 29,024.7; experiment, 29,024.3). The types of fragment ions (*c*, *z*˙, and *a*˙ ions from ECD; *b* and *y* ions from collateral collision-activated dissociation) are shown at upper right. The 183 cleavages were derived from 512 fragment ion mass values; 45 of those values (*indicated by red asterisks*) disagreed with those predicted from the sequence of the protein database by −1.0 Da, but all of them were for fragments that included Asp-10 and not Asn-31. These values differ in mass by −1.0 Da, so reversing these residues makes the predicted values agree with those found and brings the region's sequence into correspondence with related carbonic anhydrase enzymes. Reprinted with permission from Reference 117. Copyright 2003, American Chemical Society.

they enter the mass spectrometer, before refolding, greatly increases backbone cleavage. For a 1,300-residue (144-kDa) protein, this prefolding dissociation (129) caused 287 backbone cleavages, all in the first 250 residues of each terminus, whereas it caused 87 cleavages in a 229-kDa protein. For this gas-phase refolding of multiply charged cations, further H/D exchange, ECD (126), and IR photodissociation spectroscopy (127) studies, some unpublished, indicate that the α-helix is a basic conformational element of the gaseous protein ions, stabilized with a charge approximately every six residues; removing charges allows the helix to bend, then to fold, and even to form a three-helix bundle.

15. REFLECTIONS

The field of molecular MS continues to grow: There were more than 7,000 attendees at the 2010 ASMS conference (**Table 1**). It has had critical inputs from, and has made important contributions to, disciplines ranging from physics to medicine to computer science, and from physical and theoretical chemistry to organic and biochemistry. But I have viewed the field, and especially my participation in it, as analytical chemistry, and it is an appropriate honor to be included in this prestigious review of our field. In 1984, I proposed the so-called 6S criteria for analytical methods—specificity, sensitivity, speed, sampling, simplicity, and $ (130). If we except the last criterion and define some others advantageously, then the 6S performance of molecular MS by these criteria is in keeping with its impressive growth over the past century (**Table 1**).

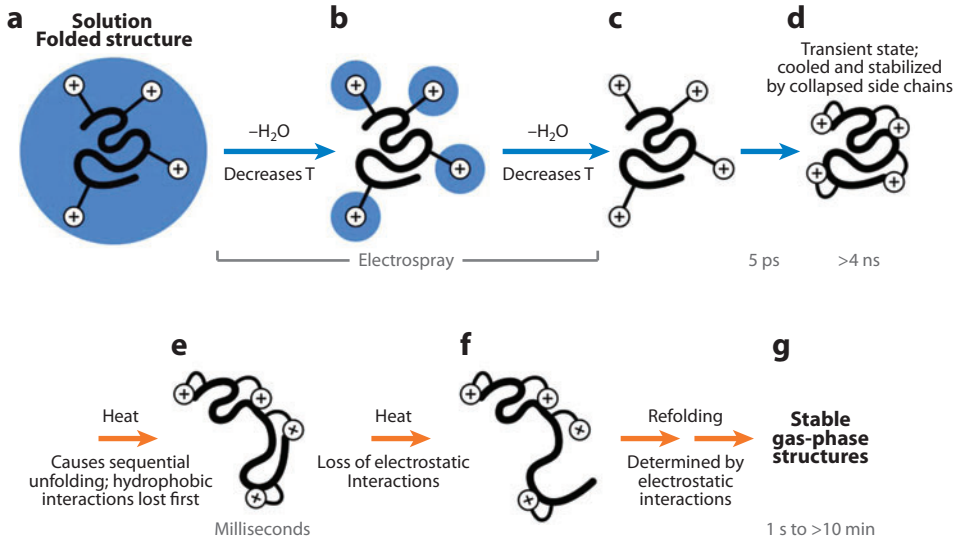

Figure 6

Stepwise evolution, after electrospray ionization, of the structure of a native or denatured protein such as cytochrome *c* or ubiquitin. On entry into the mass spectrometric vacuum system, (*a*) the protein is covered with a layer of hydrogen-bonded H_2O, which (*b*) continues to be lost with concomitant cooling. With (*c*) loss of the last water molecules, the exterior ionic functionalities (*d*) are restabilized in ~5 ps by collapse onto the dry protein. The interior of the protein undergoes thermal reequilibration in milliseconds, followed by (*e*) loss of hydrophobic bonding and then (*f*) loss of electrostatic interactions (prefolding dissociation is effected here). The transiently unfolded ions (*g*) form new noncovalent bonds within seconds; refolding of these bonds to energy minima conformers can require minutes (126). Reprinted with permission from Reference 128. Copyright 2008, National Academy of Sciences.

I have been wonderfully privileged to have had this exciting scientific experience and to have shared it with a large number of creative scientists, who are also great people and good friends. My teachers; colleagues at the Dow Chemical Company, Purdue University, and Cornell University; students; and postdoctorates have also made critical contributions. The ideas described above came mostly from discussions (the difference between shared and stolen is hazy). Funding from the Dow Chemical Company and U.S. agencies such as the National Science Foundation, the Army Research Office, and the National Institutes of Health (NIH) was critical for this expensive MS research. My special gratitude goes to the Institute of General Medical Science, NIH, which has supported me continuously since I joined academia in 1964. Best of all have been the unbelievable support and encouragement from my wife of 62 years, Tibby, and from our wonderful family. I sincerely believe that no one else could have been so lucky.

SUMMARY POINTS

1. Soon after J.J. Thomson discovered the mass spectrometer in 1910, he described its unique potential applications, such as isotope characterization and chemical analysis.
2. Molecular MS gained importance during World War II for the quantitative analysis of low-boiling hydrocarbons; petroleum applications were a dominant MS use until approximately 1960.

3. MS structure determination of hydrocarbons was unreliable; its resulting poor acceptance for all organic compounds was reversed through the development of predictable chemistry for the dissociation of their ions.
4. The development of GC/MS in 1956 greatly expanded applications of qualitative MS; molecular identifications were enhanced with reference mass spectra and algorithms for matching unknown spectra.
5. The development of MS/MS in 1966 represents another dimension of MS structural information, providing initial separation either of a mixture of molecular ions or of a molecule's fragment ions for further MS characterization.
6. MS proteomics (developed in 1990) is a prime MS/MS application in which MS-I separates peptide ion mixtures for MS-II sequencing. In top-down proteomics, MS-I separates protein molecular ions for MS-II sequencing, and ECD provides more extensive coverage than CAD.

FUTURE ISSUES

1. Top-down proteomics will become a routine technique for the characterization of larger proteins in complex mixtures given further improvements, such as two-dimensional LC preseparation, sample automation, and refined data algorithms.
2. Current studies will greatly improve our understanding of gaseous multiply charged protein conformer structures, their folding, and their effect on CAD and ECD.
3. The applicability of high-resolution top-down MS/MS for the characterization of DNA and RNA has already been demonstrated and should greatly increase.
4. High-resolution top-down MS/MS has great potential for glycomics, the characterization of carbohydrates.
5. Inexpensive, automated GC/MS systems that permit identification of toxicants (and medical treatment recommendations) through computerized databases should become common in hospital emergency rooms, with disaster response teams, and so on.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Annual Review of
Analytical Chemistry

Volume 4, 2011

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