Pharmaceutical Research, Vol. 4, No. 1, 1987

Review

Fast Atom Bombardment Mass Spectrometry and Its Application to the Analysis of Some Peptides and Proteins

Mark E. Hemling[1]

The techniques of fast atom bombardment mass spectrometry has overtaken (but not entirely replaced) field desorption mass spectrometry as the method of choice for the analysis of nonvolatile, thermally labile polar compounds. The ease with which information may be obtained on a wide variety of molecules is a result of the relative simplicity of the technique. A brief history of bioorganic mass spectrometry leading to the development of fast atom bombardment is presented, as well as a description of the method and ancillary techniques. Selected examples of its application to peptide and protein structural problems attest to the power and utility of fast atom bombardment mass spectrometry.

KEY WORDS: mass spectrometry; fast atom bombardment; peptide analysis; protein analysis.

OVERVIEW OF BIOORGANIC MASS SPECTROMETRY

Gas-Phase Ionization

Biologists and biologically oriented chemists have long provided the impetus for advances that have been made in mass spectrometry (1). The need for stable isotope analysis in the 1930s led to the development of isotope ratio instruments. In the 1950s, the utility of mass spectrometry in structural analysis was recognized and electron ionization (EI) was applied to a variety of organic and biochemical compounds. The need to reduce volatility requirements for mass analysis led to the introduction of the direct insertion probe (2), which brings the sample up to the source block prior to vaporization, thereby reducing the required sample vapor pressure from ca. 10^{-4} to 10^{-6} Torr. This technique combined with various derivatization procedures increased the scope of compounds amenable to mass spectrometry, making possible the analysis of polar, involatile compounds such as peptides and carbohydrates.

The use of derivatization has its disadvantages, however. Increased sample manipulation prior to analysis, incomplete reactions, increased analyte molecular weight, and increased spectral complexity due to the additional functional groups have led to the continued search for improved (softer) ionization techniques. The introduction of chemical ionization (CI) (3) and field ionization (FI) (4) helped to increase the yield of molecular ions (a crucial piece of information from any mass spectral experiment) but introduction of the sample in the gas phase was still necessary. This requirement was greatly reduced as analogous "in-beam" EI/CI techniques were developed (5–7). By positioning the direct insertion probe so that rapid vaporization of the sample takes place within the ion source, either within the CI reagent gas plasma or in close proximity to the ionizing electron beam, transit time of the vaporized sample molecules to the ionization region could be eliminated and hence the probability of decomposition of thermally labile compounds reduced ($K_{\text{vaporization}} > K_{\text{decomposition}}$). These techniques are referred to by a variety of names including in-beam, direct, and desorption CI or EI.

Field-Induced Sampling from Liquids/Solids

The analysis of labile, polar compounds was advanced with the introduction of several liquid/solid ionization techniques. Field desorption (FD) mass spectrometry (4), which evolved from FIMS, may be considered as a special case of field-induced sampling from liquids. According to one theory, in order to maintain a steady ion current molecules must move to the needle tips of activated emitters, where the field strength is sufficiently high for desorption. Solid organic compounds will migrate by surface diffusion only at their melting or sintering temperature (8) and therefore a heating current must generally be applied to the anode. While the field desorption/ionization process may itself impart little energy to the sample molecule and further "softening" may be a result of sampling from a liquid, the necessary heating often provides enough energy to cause some fragmentation of the analyte. For the first time, however, it was possible to obtain molecular ions from large, thermally labile compounds such as underivatized peptides. However, experimental difficulties related to the activated emitters that were used and their loading limited the success of FD in the hands of all but a few skilled spectrometrists.

Several other methods of sampling ions from a liquid phase have been developed recently. Thermospray ionization (9) and electrospray ionization (10,11) have often been reported in conjunction with interfaces for liquid chromatog-

[1] Physical & Structural Chemistry, SmithKline & French Laboratories, P.O. Box 7929, Philadelphia, Pennsylvania 19101.

0724-8741/87/0200-0005$05.00/0

raphy/mass spectrometry (LC/MS) but may also be used as methods for introduction and ionization of individual compounds. Electrohydrodynamic ionization (EH) mass spectrometry also shows potential for interfacing liquid chromatography to mass spectrometry (12). All three methods rely on high electrical fields to effect the desorption of ions (often solvated) from a liquid matrix resulting in ions with low internal energies and, hence, mass spectra with few, if any, fragment ions.

Particle-Induced Desorption

The introduction of ^{252}Cf plasma desorption (PD) mass spectrometry by Macfarlane and co-workers in 1974 (13) was a leap forward in sample ionization methodology. Many of the aforementioned techniques require heating of the sample on a slow or prolonged (seconds) time scale. Macfarlane *et al.* reasoned that application of heat on a much shorter (10^{-6}- to 10^{-12}-sec) time scale might induce desorption before the sample molecule has time to absorb energy into unstable vibrational modes. Such energy could be imparted by the high-energy fission fragments from ^{252}Cf. One of the initial reports (14) demonstrated successful analysis of a number of compounds including cyanocobalamin ($[M - H - HCN]^-$, *m/z* 1327) and a gramicidin A mixture ($[M + Na]^+$, *m/z* 1904, 1918). The attention of biochemists should have been seized in 1981 by the analysis of a nucleotide dimer at *m/z* 12,637 (15). The molecular weight record has since been increased to ca. 25,000 daltons (Da) with the analysis of porcine trypsin (16). Desorption has also been induced by 90-MeV ^{127}I ions from a nuclear accelerator (17). The ability to determine molecular weights for the components of mixtures of polypeptides and proteins that could not be separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis or high-performance gel permeation chromatography has also been demonstrated with a precision that is ca. 2 orders of magnitude better than that of either of the chromatographic methods (18).

The use of time-of-flight (TOF) mass analysis required by these techniques results in a high sample efficiency and a theoretically unlimited mass range, but the short ion burst produced by a fission fragment precludes implementation on the scanning-type mass spectrometers that are most widely used. Until very recently, PDMS instrumentation was not commercially available. Widespread implementation of a technique with similar potential due to its ability to deposit a large amount of energy in a short time period, laser desorption (LD) mass spectrometry (19), has likewise not occurred, even though the applicability of LDMS has been demonstrated for such biologically important compounds as oligosaccharides, glycosides, and nucleotides (19).

A technique that may be implemented on either TOF or scanning-type mass spectrometers and that uses keV ions (e.g., Ar, Xe, Cs) to deposit energy to a solid sample is secondary ion (SI) mass spectrometry (20,21). Two types of SIMS experiments are possible, depending on the ion flux used. Dynamic SIMS (high primary ion current, ca. 10^{-6} A/cm^2) yields high secondary ion production and fragmentation due to sample damage from multiple primary ion hits at a given site during the experiment. This mode is frequently used for depth profiling studies. Static SIMS (low primary ion current, $<10^{-9}$ A/cm^2) statistically prevents primary ions from hitting the same spot during an experiment. These conditions reduce fragmentation relative to the molecular ion but also result in very low secondary ion fluxes. Useful spectra have been obtained for a number of biological compounds including saccharides (22) and nucleotides (23) under static SIMS conditions. Low secondary ion currents, instrumental difficulties in directing a primary ion beam into the high-voltage source of a magnetic sector instrument, and charging of the sample target have limited the widespread application of SIMS.

FAST ATOM BOMBARDMENT MASS SPECTROMETRY

In 1981, Barber and co-workers at the University of Manchester Institute of Science and Technology (UMIST) introduced (24,25) a technique that combined some of the features of particle desorption and liquid sampling ionization methods. The technique, somewhat inappropriately termed fast atom bombardment (FAB) mass spectrometry[2] (*vide infra*), is most similar to SIMS in that it involves bombardment of the sample by keV particles. Barber and co-workers chose to use atoms rather than ions as the energetic species, which they suggested would help to eliminate charging effects and also simplify conversion of magnetic sector instruments for FAB. The key contribution of the UMIST group was the use of a viscous liquid matrix in which the sample is dispersed. This allowed the use of high primary beam fluxes without the concomitant surface damage effects observed in dynamic SIMS. The use of a liquid matrix imparts the softening effects observed in the liquid sampling techniques, providing high molecular ion intensities from labile molecules for prolonged periods of time (ca. 5–30 min.). The matrix also provides the characteristic FAB spectral background consisting of ionized matrix clusters (e.g., nG + H, G = glycerol) and the peak-at-every-mass chemical noise, both of which can be useful in calibrating the spectrum.

Method of Fast Atom Bombardment Mass Spectrometry

The Matrix and the Sample

The critical aspect of a FABMS experiment is the matrix solvent used to disperse the sample. [A recent review discusses matrices and their role in the sputtering process (27) (Fig. 1).] Glycerol was the initial solvent of choice (28), possessing the required properties of low vapor pressure under high vacuum conditions and the ability to solubilize highly polar compounds. While glycerol still sees widespread use, a number of matrices have been developed for various applications. Tetraethylene glycol (tetragol) may be used in cases where glycerol is too polar (27). Thioglycerol and a mixture of dithiothreitol and dithioerythritol (DTT/DTE; 3:1) are particularly effective for the analysis of peptides (29,30), oligosaccharides (31), and corticosteroids (32) and have also been useful with some porphyrins (33). Tetramethylenesulfone and the dimethyl ether of tetragol have found application with less polar and acid sensitive com-

[2] The terms FABMS and liquid SIMS are essentially synonymous in the literature. The issue of nomenclature has recently been discussed (26).

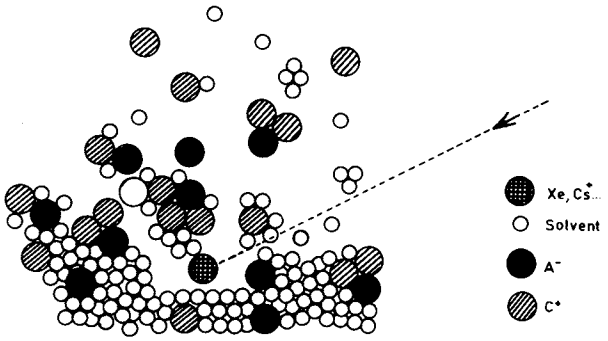

Fig. 1. Schematic representation of the sputtering of a solution of an electrolyte. (Reproduced with permission from Ref. 27.)

pounds (34). The analysis of glycosphingolipids in the negative ion mode has been facilitated by the introduction of basic matrices such as di- and triethanolamine, sometimes with the addition of substituted ureas (35,36), and by triethylenetetramine (TETA) (37). Porphyrins and corrins, which undergo hydrogenation in glycerol upon bombardment, have been successfully analyzed in *o*-nitrophenyl octyl ether (NPOE), while polynitriles of the same basic structure, which are not readily soluble in NPOE, yield to analysis in a *m*-nitrobenzyl alcohol (3-NOBA) matrix (38). The recent development of a cooled sample introduction probe (39) and a continuous-flow probe (40) will broaden the range of matrices compatible with the FABMS technique and hence the types of compounds amenable to analysis.

It is often useful to modify the matrix in order to increase sensitivity or to form known adducts. Acids [HCl, oxalic, acetic, etc. (41)] or bases [urea (36)] may be added to promote formation of ionic species prior to desorption in the positive and negative ion modes, respectively. Traces of ions such as sodium and potassium are used to form cation adducts of the analyte, either to increase sensitivity for the molecular ion or to confirm its presence by observation of the appropriate mass shift as has been done in FDMS (42). However, higher levels of salts, particularly the presence of involatile buffers or ion-pairing reagents in chromatographically prepared samples, can completely suppress ionization of the analyte of interest.

Microderivatizations may be performed in the matrix immediately prior to analysis. This has proven to be useful for peptides, where the N-terminal sequence ions may be distinguished by forming an equimolar mixture of $^1H_3$-acetyl and $^2H_3$-acetyl derivatives of the peptide sample, which will yield doublets separated by 3 Da for the N-terminal sequence ions (43). Derivatization historically has been used to decrease polarity and increase volatility of the analyte. However, compounds that are ionic (salts) or readily able to form ions (acids, bases) show increased sensitivity toward desorption ionization (44,45). Busch *et al.* have advocated the use of "reverse derivatization," i.e., derivatization with the intent of making the analyte more polar or ionic and less volatile, in preparing samples for desorption mass spectrometry (44). This idea has been applied in the development of a procedure for the quaternization of peptides (46).

Aside from possible derivatization procedures, sample preparation involves dissolving the sample in a suitable volatile solvent and adding an appropriate amount of this solution to 0.3–3 μl of matrix on the target in order to obtain a loading of 1–10 μg of analyte. Linear working curves for the molecular ion have been generally observed from the microgram to the nanogram level, near the background-determined limit of detection (29). Saturation often occurs between 1 and 20 μg and quantitative analyses are best done using isotopically labeled internal standards (47).

The Bombarding Particles

The fast atom gun used in FABMS is essentially the same gun that is used to produce ions for SIMS, but operated at a higher pressure and usually equipped with a deflector plate (48). The higher pressure results in neutralization of the inert gas ions by electron capture or charge exchange and the deflector plate removes residual ions from the beam. The guns are normally operated at 3–10 kV, producing ions that are nominally of said energy. Ligon observed that the ion output of such a gun displays a broad energy spread, from 1 to 30 keV, and proposed that a similar distribution exists for the neutralized species (49). At first, FABMS appeared to avoid the problems of SIMS through the use of a neutral beam of atoms, facilitating implementation on sector instruments. After it became apparent that the most important contribution of FABMS was the enhanced sample lifetime and ion current due to the liquid matrix used to disperse the sample, work began to optimize the particle beam conditions for FABMS. Upon changing the neutral beam from argon to xenon, Hunt *et al.* (50) found a three- to fivefold increase in sensitivity. Later, Rudat and McEwen (51) and Aberth *et al.* (52) noted enhanced high mass sensitivity with a cesium ion gun compared to an argon atom gun and concluded that the charge state of the primary beam had no influence on the production of secondary ion spectra from viscous liquid matrices. Stoll *et al.* (53) found a 10-fold increase in sensitivity vs argon when using a mercury ion beam and later investigated focused and unfocused liquid metal ion guns more extensively, finding the following relative sensitivities for various primary beam species—Ar(1):Ga(10):In(40):SiAu(50); the present maximum appears to be from the eutectic SiAu mixture (53).

Other Instrumental Parameters

A third, lesser contribution of the inventors of FABMS was the use of a 70° angle of incidence (θ) for the particle beam. The UMIST group empirically determined that maximum sensitivity is achieved at this glancing angle. Sigmund, at a symposium on fast atom and ion-induced mass spectrometry (55), pointed out that sputtering yields theoretically vary with cosθ. However, above 75° the particles penetrate only shallow layers and much energy is lost to high-energy particles that are not detected. The UMIST group also noted that fragmentation increased as the angle of incidence is decreased. This is in agreement with the Monte Carlo calculation by Magee, which showed that more low-energy collision cascades reach the surface to produce sputtering events at a greater angle of incidence (56).

Various materials, including stainless steel, copper, and polyimide-coated stainless steel, have been used as target materials on which the sample and matrix are introduced

into the mass spectrometer ion source (41). The target material has little effect on the spectrum except copper, which occasionally contributes background or adduct ions. The insulating polyimide target showed no deleterious charging effects from an incompletely neutralized atom beam. Targets are generally of two forms—a ribbon of metal ca. 2 × 7 mm and a solid round target with a diameter of ca. 2.5 mm.

Ancillary Techniques

The prolonged spectral lifetime and high secondary ion intensity of FABMS permit the use of auxiliary mass spectral techniques. High resolution capabilities allow accurate mass measurements, and several methods have been developed for use with FABMS. Masses for ions recorded on photoplates may be accurately measured using either the matrix ions (e.g., glycerol) or an overlaid Fomblin (perfluoropolyphenyl ether) EI spectrum for reference masses (57). Signal intensity is often sufficient that peak matching may be done, with internal standards that are applied with the sample to the matrix on a single target (58) or with external standards applied separately on a split target (59,60). Multichannel averaging (MCA) an improve measurements, especially for weak signals, and has been employed in conjunction with EI calibration (61) or with FAB calibration using either internal standards (62) or external standards on a split target (60). Several scanning techniques have been implemented employing accelerating voltage scanning over limited mass ranges (ca. 200 Da) (63) or magnetic scanning over more extended mass ranges (ca. 750 Da) with correction for magnetic drift (64).

Various MS/MS techniques are possible including MIKES, linked scanning, and collision-induced dissociation (CID) experiments. Stabilities of cationic transition metal complexes were found to depend on the transition metal center and the size of the equatorial alkyl substituent by FABMS/MS (65). Artifact peaks in the spectrum of cobalamin were determined by FABMS/MS to be due to the intermolecular transfer of a cobalt atom from one molecule to the phosphate group of another (66). Mixture analysis has been demonstrated for various lipids including cationic (67) and anionic (68) surfactants and zwitterionic ornithine-containing lipids, for which positive- and negative-ion CID MS/MS yielded complementary information (69). MS/MS can be especially useful for extracting sequence information and has been used efficaciously in the analysis of peptides (30,50,70,71), carbohydrates (72), and nucleotides (73–76). While low resolution is sufficient for many applications, permitting the use of triple quadrupole analyzers (50), high resolution is required for certain analyses and the application of HRFABMS/MS has been demonstrated (77).

A natural combination of techniques for separation and analysis of nonvolatile, thermally labile, polar compounds has resulted in the recent development of LC/FABMS employing a moving belt interface. The first report demonstrated the separation and analysis of uridine and adenosine in which the glycerol matrix was added to the LC solvent system (78). Oligosaccharides and peptides have been analyzed both with and without a glycerol matrix (79); sensitivity was found to be at least a factor of 10 better when the matrix was used. The promise of the technique has been further proven by the analysis of larger peptides to ca. 1800 Da and the analysis of the partial hydrolysis mixture of antiamoebin I (80). Improvements in the method of sample deposition on the belt have improved sensitivity and chromatographic integrity (81). Recent reports of continuous-flow introduction probes for FABMS hold some potential for coupling with the low flow rates of microbore and capillary LC (40,82).

Applications

Over 600 papers describing FABMS and its applications have appeared in the literature since its introduction in 1981. A number of reviews and overviews have since been published (27,28,45,61,83–87). FABMS has been particularly useful for the analysis of peptides and proteins, providing molecular weight information for large peptides such as the bee venom peptide melittin (2845 Da) (88), glucagon (3841 Da) (88), adrenocorticotropic hormone (ACTH, 4538 Da) (89), the sweet proteins monellins (ca. 5500 Da) (90), insulins (ca. 5700 Da) (89,91), and human and bovine proinsulins (ca. 9100 Da) (92,93), and often providing both molecular weight and sequence information for smaller peptides (43,58,94,95). A variety of sugar-containing compounds such as glycopeptides (96,97), complex oligosaccharides (98) including the aminoglycoside antibiotics kanamycins (99), glycosphingolipids and gangliosides (36,37), and glycosides and their sulfates (100–103) have been analyzed. Compounds containing the phosphate group have been studied including phosphatidylcholines (104,105), the phosphoglycolipid antibiotic pholipomycin (106), and nucleotides (73,107,108). Many other classes of bioorganic compounds have proven to be amenable to FABMS: steroidal sulfates (109), penicillins (110), and antibiotic monobactams (111) and macrolides (112). Organometallic complexes of biochemical interest, e.g., cobalamines (113), bleomycin complexes (96), hydroxamate-containing siderophores (114), and cationic technetium complexes (115), have been the subjects of several reports.

FABMS has also been applied in areas that are not of direct biochemical interest. FABMS has been useful in fossil fuel analysis for the identification of nitrogen-containing compounds (116); a liquid–metal matrix facilitated analysis of polycyclic aromatic compounds (117); azosulfonic acid dyestuffs (118), diquaternary ammonium salts (119,120), and simple salts (121) have been studied, as have the redox processes of glycerol solutions of inorganic salts, organometallics, and cationic dyes (122). High mass capability for inorganic compounds has also been demonstrated with the analysis of large polyoxoanion catalysts (ca. 2500–7500 Da) (123,124).

SEQUENCING OF PEPTIDES AND PROTEINS

The remainder of this review addresses the application of FABMS to peptides and proteins. Because of the many papers in this area already in the literature, only a necessarily biased selection of these references is presented in order to convey the contribution that FABMS can make in solving peptide and protein structures and to assess the potential for sequencing proteins de novo. For a more comprehensive survey of the literature, see pertinent sections of the biennial reviews of mass spectrometry (125).

Since the introduction of FABMS in 1981, peptides and

their conjugates have been the focus of much of the work published on this technique. Many of the early reviews and overviews (43,58,94) were quick to point out that both molecular weight and sequence information could be obtained from many of the peptides of known structure that were studied. While such information did allow the revision of several previously assigned peptide structures, the rate of assignment of new structures has not drastically increased. A recent evaluation of the technique vis-à-vis peptide sequence determination (126) provides several reasons for this. The FAB spectra obtained for unknowns frequently contain sequence ions that are of low abundance. The ubiquitous presence of alkali metal ions in unknowns, while allowing confirmation of the molecular weight, seems to result in the low abundance of sequence information. The many different fragmentation pathways that may be followed (Fig. 2) further complicate interpretation. Roepstorff concludes that a general procedure for peptide sequencing involves FAB with on-probe acetylation, manual microsequencing, and EIMS. Other generalized procedures for unknown peptides also rely on a variety of techniques including hydrolysis, GC, and EI- and CI-MS in addition to FABMS (128). Several examples of the kinds of procedures required for the sequence assignment of novel or unknown peptides are discussed below.

Techniques in the Analysis of Peptides and Proteins

The analysis of peptides and small proteins of unknown molecular weight is most readily accomplished by determining the approximate molecular weight using one of several methods of fast, wide mass range survey scanning (129) at a very low resolution for highest sensitivity. An accurate mass measurement (±0.5 Da) may then be made by slower scanning over a narrower mass range at the desired mass resolution. The value of fully resolved molecular ions at higher mass is questionable, however, as the contribution of higher isotopic species to the molecular ion envelope becomes predominant (130). While full resolution of molecular ion envelopes may be a good mass spectrometric exercise and may be useful in testing instrument performance, maximization of sensitivity without loss of information will be particularly important for the development of MS/MS techniques for proteins.

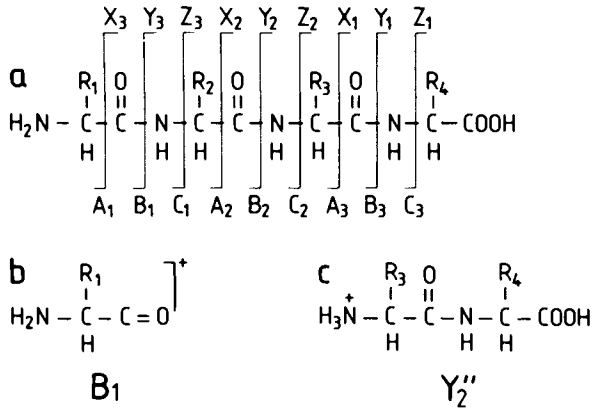

Fig. 2. Fragmentation nomenclature for peptide sequence ions. (Reproduced with permission from Ref. 127.)

Numerous techniques, methods, and procedures have been implemented in various labs to maximize the information obtained from FAB mass spectra of peptides and proteins. The use of 1:1 $^{1}H_3/^{2}H_3$-N-acetyl derivatives for distinguishing N-terminal from C-terminal sequence fragment ions has already been discussed. Direction of fragmentation has been demonstrated by the introduction of a dansyl group at the N terminus of the peptide, eliminating C-terminal fragment ions from the spectrum (131). Unequivocal assignment of N-terminal ions may be achieved by labeling with 2-bromo-5-(dimethylamino)benzylsulfonyl chloride; the spectra are complicated by debromination ions, however, and, for peptides over 10 amino acids, the ^{13}C contribution to the isotope pattern is significant, reducing the ability to distinguish N-terminal from C-terminal ions. Determination of the number of acidic residues in a peptide or protein can be made by glycinamidation and examination of the products by FAB for increases in mass (132). This modification was also found to increase the efficiency of tryptic digestion by neutralizing negative charge sites in the protein.

The oxidation state of cysteine-containing peptides or proteins is often important for biological function. The FAB spectra of disulfide-containing peptides are often characterized by a changing ratio of oxidized to reduced forms, the rate of which may be affected by the matrix being used. Difficulty in determining the extent of reduction of potential disulfide linkages in the native molecule may be influenced most by intrinsic molecular properties of the peptide or protein (133). For example, a sample of vasopressin in glycerol was observed to undergo reduction on the probe tip (Fig. 3), while oxytocin in the more reducing dithiothreitol/dithioerythritol (DTT/DTE) matrix was observed to be stable to reduction. Salmon calcitonin has also been demonstrated to be insensitive to reduction by the atom/ion beam, the liquid matrix, or the metal of the probe tip (134). Analysis of the extent and location of S–S bridging is important for total characterization of the protein structures and of particular importance for determining whether a recombinant protein is folded the same as the native protein. Chemical or proteolytic digestion of proteins before and after reduction of the disulfide linkages provides a means of assigning locations using modification of standard FAB mapping techniques (*vide infra*) (135).

Enzymatic hydrolysis using carboxypeptidase Y or leucine aminopeptidase has been used in conjunction with FABMS to provide a mass-dependent rather than a time-dependent analysis for peptide sequencing, alleviating the problems encountered when multiple identical residues appear in sequence (136). Analyses may be done by direct deposition of an aliquot of the digestion mixture into the matrix on the probe with no apparent interference from the exopeptidase. Continuous analysis of the mixture has been effected using a belt LC/MS interface (137) and should be possible with the recently developed continuous liquid introduction FAB probe (82) to provide sequence information as a function of both time and mass.

Assignment and Peptide Structures

Structural assignment is aided considerably by the recognition and analysis of analogous compounds. Invertebrate neuropeptides, MI and MII (Fig. 4), isolated from the cor-

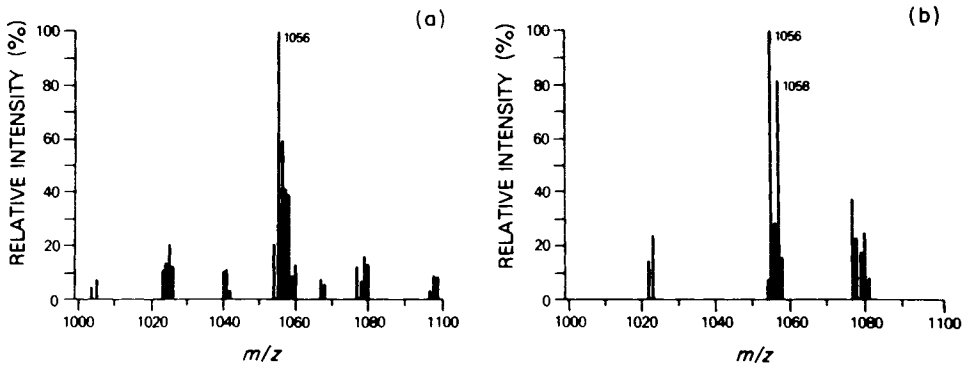

Fig. 3. The $[M + H]^+$ ion region of the positive-ion FAB mass spectra of 1.0 nmol of vasopressin (a) after 31 sec and (b) after 93 sec of continuous bombardment, using a glycerol matrix on a gold-plated copper sample stage. (Reproduced with permission from Ref. 133.)

pora cardiaca of the American cockroach *Periplaneta americana* (138), adipokinetic hormones (AKH) II from the locusts *Schistocerca nitans, S. gregaria,* and *Locusta migratoria* (139), and AKH from the tobacco horn worm *Manduca sexta* (140) were recognized as being similar to AKH I from locusts and to red pigment-concentrating hormone (RPCH) from prawns. Sequences of the N- and C-terminally blocked peptides were assigned by comparison of the normal FAB and B/E linked scan spectra (138–140) and accurate mass measurement data (138). The assignments for the natural peptides were further confirmed by comparison of the MS, HPLC, and bioactivity data from the synthetic peptides (138,139).

The structures of three cyclic peptides from the tunicate *Lissoclinum patella* were assigned and the structure of the analogous ulicyclamide was revised on the basis of positive- and negative-ion FABMS and other data (141). HREIMS provided molecular formulas and detailed decoupling of the ^1H NMR suggested amino acid compositions for the three peptides which resembled that of ulicyclamide. Extensive accurate mass measurements on the FABMS fragment ions of the selective hydrolysis product of each peptide provided unique assignments and, together with GC and GC/MS data, allowed assignment of total structures for the four peptides. Three of these structures have recently been revised (141b) based on additional NMR data and information obtained by FABMS/MS that was not available in the normal FAB mass spectrum. These examples illustrate both the power of NMR and FABMS when used in combination and the caution that must be exercised when interpreting data, especially those on cyclic peptides.

As peptides get larger, with the often concurrent de-

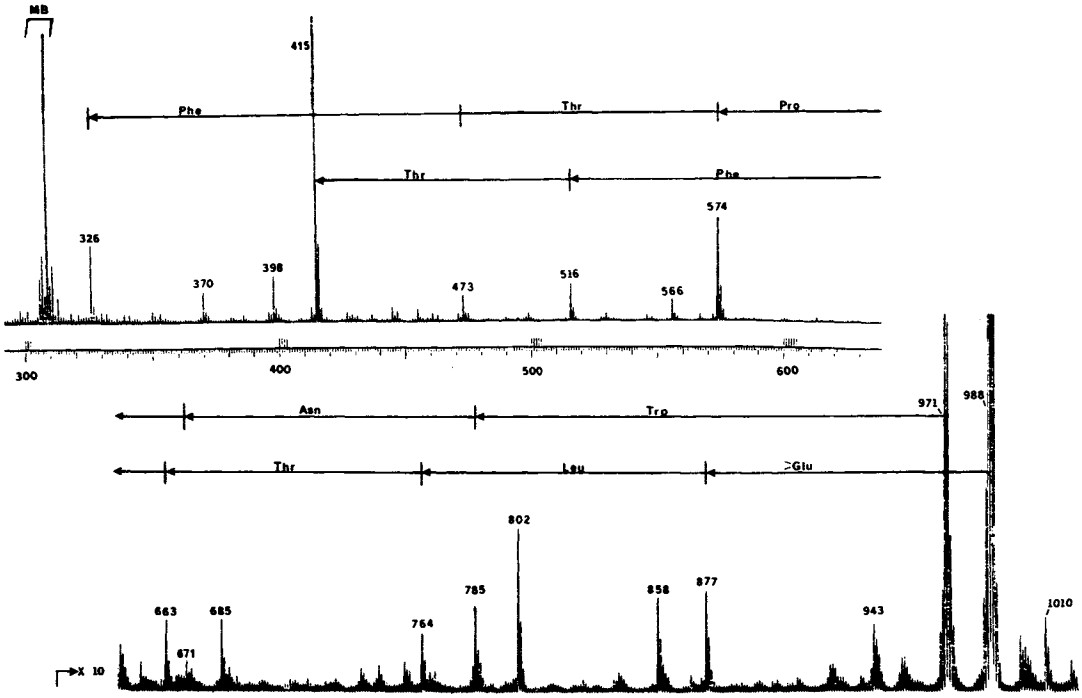

Fig. 4. FAB mass spectrum in DTT/DTE of M II, Pca–Leu–Thr–Phe–Thr–Pro–Asn–Trp–NH_2, isolated from *P. americana*. (Reproduced with permission from Ref. 138.)

crease in sequence-related FABMS fragment ions, the methods required for analysis begin to change. One method that relies only on positive- and negative-ion FABMS and linked scan experiments requires the generation of detailed tables of successive series of possible sequence ions once a molecular weight has been established (142). After potential terminal amino acids have been determined using the first table, a new table is required for the next step of analysis. While this method is tedious, the tables could be readily generated by computer, and with the addition of decision-making or weighting processes, the entire procedure might even be automated.

Other methods for large peptides rely on additional sample manipulation prior to mass spectral analysis. The assignment of the sequence of the 26 amino acid delta hemolysin isolated from a canine strain of *Staphylococcus aureus* was accomplished by a combination of amino acid composition analysis, enzymatic digestions, HPLC, FABMS, and FABMS/MS (143). Peptides generated by elastase or *S. aureus* protease digestions were fractionated by HPLC, converted to their 1:1 $^1H_3/^2H_3$-0-methyl esters, and sequenced by collision-induced dissociation of their FABMS molecular ion doublets. The peptide sequences thus generated were overlapped to provide a tentative structure of canine delta hemolysin that agreed with the FAB-determined weight for the intact polypeptide. The structure was confirmed by FAB molecular weight analysis of peptides generated by trypsin, chymotrypsin, and *S. aureus* protease digestion of the polypeptide and mapping of those weights into the proposed sequence.

Confirmation/Correction of Protein Sequences

Methods for verifying protein sequences derived from the corresponding DNA sequences, under such names as "FAB-mapping" (144a), "digit printing" (144b), and "protein fingerprinting" (144c), have been developed in various laboratories. In their most basic form these procedures involve either chemical or enzymatic digestion of 5–50 nmol of the protein, FAB mass analysis of the resultant mixture, and comparison of experimentally determined molecular weights with those calculated from the proposed sequence. In the simplest case all observed signals will map into the DNA-derived sequence, providing confirmation of a certain percentage of the protein structure. Frequently not all of the expected molecular ions are observed because of, among other possible factors, differences in surface activity (145) or *p*Ka's or the presence of interfering matrix peaks; HPLC fractionation of the mixture may help to alleviate these effects (146). The gross mixture or HPLC subfractions may be subjected to one or more steps of manual Edman degradation to confirm the mapping assignments or to sort out ambiguous assignments. The level of coverage can often be increased by repeating the procedure with a different digestion

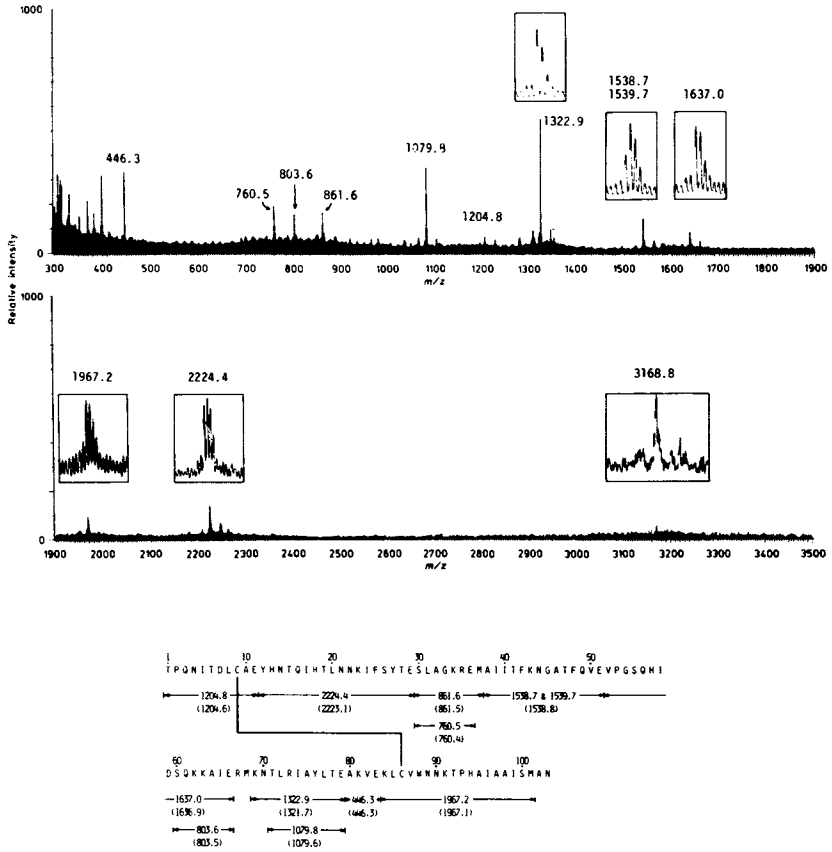

Fig. 5. FAB mass spectrum of a *S. aureus* protease V8 digest of the CNBr-treated B subunit of *V. cholerae* classical biotype Inaba 569B toxin. Insets show 15 accumulated scans over limited mass ranges. (Reproduced with permission from Ref. 150.)

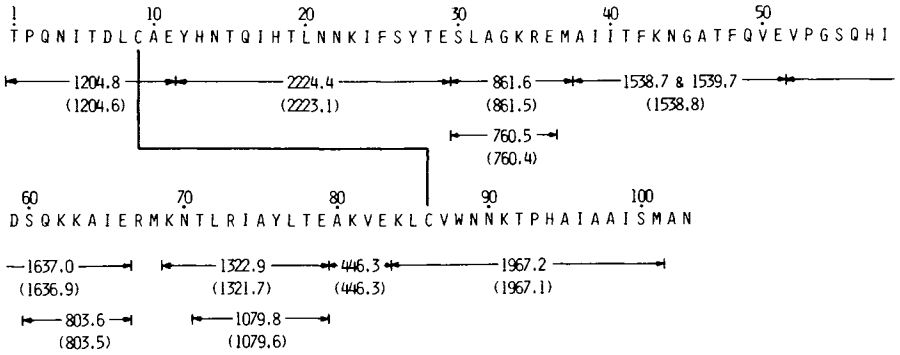

Fig. 6. Amino acid sequence of the B subunit of *V. cholerae* classical biotype Inaba 569B toxin. Numbers not in parentheses show *m/z* values observed in Fig. 5; numbers in parentheses show theoretical mass values calculated from the amino acid sequence. (Reproduced with permission from Ref. 150.)

strategy until the desired coverage is achieved. In some cases errors will become apparent which can often be corrected by reexamination of the DNA sequence data (146).

A number of protein sequences have been analyzed by these procedures including confirmation of the structures of both subunits of *Escherichia coli* glycyl-tRNA synthetase (147); identification of the blocked N terminus of ATPase inhibitor from beef heart mitochondria and correction of the protein sequence (148); revision of the primary structure of neocarzinostatin and reassessment of homology with macromomycin and actinoxanthin (149); confirmation of the sequence of the B subunit of *Vibrio cholerae* classical biotype Inaba 569B toxin (Figs. 5 and 6) and comparison to the sequence predicted from the nucleotide sequences of the genes of El Tor biotype strains 62746 and 2125 toxins (150); verification of the amino acid sequence of protein S, a protein produced only during differentiation of *Myxococcus xanthus* (151); protein fingerprinting of normal and variant haemoglobins (144b,c); and verification of the sequence and assignment of the disulfide bridge of recombinant human interleukin-2 expressed in *E. coli* and in the Jurkat-111 cell line (152,153). These and other examples rely heavily on other information in order to establish the primary structure of the protein, either from the nucleotide sequence or from sequencing portions of the protein using microsequencing or more traditional mass spectrometric techniques.

Potential for Assignment of Protein Structure

The potential for sequencing of polypeptides and proteins of unknown structure by FABMS lies primarily in two techniques—tandem mass spectrometry (MS/MS) and liquid chromatography mass spectrometry (LC/MS). The power of CID MS/MS in reducing matrix-related chemical noise and increasing observable fragment ions is well documented (138–143,154). The potential for sequencing by MS/MS has probably been most clearly demonstrated for the case of canine delta hemolysin, the 26-amino acid polypeptide discussed above (143). Although no spectra were presented, the tabular data indicated that the results of many MS/MS experiments on HPLC fractions from two different protease digests of polypeptide provided many sets of overlapping signals sufficient, with other data (amino acid composition, molecular weight), to define a unique sequence for the molecule. The major drawback with this procedure is the necessity to handle a large number of HPLC fractions, a laborious task when trying to analyze large proteins. The ability to obtain reproducible, high-quality daughter ion spectra from molecular ions in mixtures will determine the potential of this technique in competing with other sequencing methods.

An alternative to MS/MS for the analysis of protease digest mixtures is LC/FABMS. Sample handling problems associated with large numbers of LC fractions are eliminated and the introduction of peptides into the ion source as individual components or mixtures of only two or three components has been demonstrated to provide sequence information for each component (155). By using a belt interface, the matrix may be eliminated because the moving belt provides a continually renewed surface. This in turn reduces chemical noise, allowing assignment of fragment ions at lower mass. In comparison to MS/MS experiments, sequence ion signals are stronger since losses due to scattering and inefficient collisional activation are avoided. Sensitivity is similar to that for the protein mapping techniques—at the ca. 10-nmol level. The lower relative cost of LC/MS vs MS/MS should make it a reasonable alternative for peptide and protein sequence analysis. Further work needs to be done to demonstrate the general applicability of these techniques but the initial results are promising.

ACKNOWLEDGMENTS

The author wishes to thank Drs. Steven Carr and Kenneth Straub and Mr. Gerald Roberts for useful criticism of the manuscript.

REFERENCES

1. G. Waller (ed.). In *Biochemical Applications of Mass Spectrometry*, Wiley-Interscience, New York, 1972, pp. xii–xiv.
2. R. I. Reed. *J. Chem. Soc.* 3432–3436 (1958).
3. M. S. B. Munson and F. H. Field. *J. Am. Chem. Soc.* 88:2621–2630 (1966).
4. H. D. Beckey. *Principles of Field Ionization and Field Desorption Mass Spectrometry*, Pergamon, Oxford, 1977.
5. A. Dell, D. H. Williams, H. R. Morris, G. A. Smith, J. Fenney, and G. C. K. Roberts. *J. Am. Chem. Soc.* 97:2497–2502 (1975).

6. M. Ohashi, K. Tsujimoto, and A. Yasuda. *Chem. Lett.* 439–440 (1976).
7. M. A. Baldwin and F. W. McLafferty. *Org. Mass Spectrom.* 7:1353–1356 (1973).
8. H. D. Beckey. *Principles of Field Ionization and Field Desorption Mass Spectrometry*, Pergamon, Oxford, 1977, pp. 22–24.
9. C. R. Blakley, J. J. Carmody, and M. L. Vestal. *J. Am. Chem. Soc.* 102:5931–5933 (1980).
10. M. Dole, R. L. Hines, L. L. Mack, R. C. Mobley, L. D. Ferguson, and M. B. Alice. *Macromolecules* 1:96–97 (1968).
11. C. M. Whitehouse, R. N. Dreyer, M. Yamashita, and J. B. Fenn. *Anal. Chem.* 57:675–679 (1985).
12. D. S. Simons, B. N. Colby, and C. A. Evans. *Int. J. Mass Spectrom. Ion Phys.* 15:291–302 (1974).
13. D. F. Torgerson, R. P. Skowronski, and R. D. Macfarlane. *Biochem. Biophys. Res. Commun.* 60:616–621 (1974).
14. R. D. Macfarlane and D. F. Torgerson. *Science* 191:920–925 (1976).
15. C. J. McNeal and R. D. Macfarlane. *J. Am. Chem. Soc.* 103:1609–1610 (1981).
16. B. Sundqvist, P. Roepstorff, J. Fohlman, A. Hedin, P. Hakansson, I. Kamensky, M. Lindberg, M. Salehpour, and G. Sawe. *Science* 226:696–698 (1984).
17. P. Hakansson, I. Kamensky, B. Sundqvist, J. Fohlman, P. Peterson, C. J. McNeal, and R. D. Macfarlane. *J. Am. Chem. Soc.* 104:2948–2949 (1982).
18. P. Roepstorff, B. Sundqvist, J. Fohlman, P. Hakansson, I. Kamensky, M. Lindberg, A. Hedin, and G. Save. Abstr., 32nd Annu. Conf. Mass Spectrom. Allied Topics, San Antonio, Tex., May 27–June 1, 1984, pp. 32–33.
19. M. A. Posthumus, P. G. Kistemaker, H. L. C. Meuzelaar, and M. C. Ten Noever de Brauw. *Anal. Chem.* 50:985–991 (1978).
20. A. Benninghoven and W. Sichtermann. *Anal. Chem.* 50:1180–1184 (1978).
21. R. J. Day, S. E. Unger, and R. G. Cooks. *Anal. Chem.* 52:557A–572A (1980).
22. H. Kambara and S. Hishida. *Anal. Chem.* 53:2340–2344 (1981).
23. A. Eicke, W. Sichtermann, and A. Benninghoven. *Org. Mass Spectrom.* 15:289–294 (1980).
24. M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler. *J. Chem. Soc. Chem. Commun.* 325–327 (1981).
25. D. J. Surman and J. C. Vickerman. *J. Chem. Soc. Chem. Commun.* 324–325 (1981).
26. A. L. Burlingame, T. A. Baillie, and P. J. Derrick. *Anal. Chem.* 58:165R–211R (1986).
27. E. De Pauw. *Mass Spectrom. Rev.* 5:191–212 (1985).
28. (a) D. H. Williams, C. Bradley, G. Bojesen, S. Santikarn, and L. C. E. Taylor. *J. Am. Chem. Soc.* 103:5700–5704 (1981); (b) M. Barber, R. S. Bordoli, G. J. Elliott, R. D. Sedgwick, and A. N. Tyler. *Anal. Chem.* 54:645A–657A (1982).
29. W. D. Lehmann, M. Kessler, and W. A. Konig. *Biomed. Mass Spectrom.* 11:217–222 (1984).
30. J. L. Witten, M. H. Schaffer, M. O'Shea, J. C. Cook, M. E. Hemling, and K. L. Rinehart, Jr. *Biochem. Biophys. Res. Commun.* 124:350–358 (1984).
31. V. N. Reinhold and S. A. Carr. *Mass Spectrom. Rev.* 2:153–221 (1983).
32. J. Belanger, B. A. Lodge, J. R. J. Pare, and P. Lafontaine. *J. Pharm. Biomed. Anal.* 3:81–86 (1985).
33. L. Kurlansik, T. J. Williams, J. M. Strong, L. W. Anderson, and J. E. Campana. *Biomed. Mass Spectrom.* 11:475–481 (1984).
34. K. L. Busch, B.-H. Hsu, K. V. Wood, and R. G. Cooks. *J. Org. Chem.* 49:764–769 (1984).
35. K. Harada, M. Suzuki, and H. Kambara. *Org. Mass Spectrom.* 17:386–391 (1982).
36. M. Arita, M. Iwamori, T. Higuchi, and Y. Nagai. *J. Biochem.* 94:249–256 (1983).
37. M. E. Hemling, R. K. Yu, R. D. Sedgwick, and K. L. Rinehart, Jr. *Biochemistry* 23:5706–5713 (1984).
38. J. Meili and J. Seibl. *Org. Mass Spectrom.* 19:581–582 (1984).
39. A. M. Falick, F. C. Walls, and R. A. Laine. Abstr. 34th Annu. Conf. Mass Spectrom. Allied Topics, Cleveland, Ohio, June 8–13, 1986, pp. 377–378.
40. Y. Ito, T. Takeuchi, and D. Ishii. *J. Chromatogr.* 346:161–166 (1985).
41. S. A. Martin, C. E. Costello, and K. Biemann. *Anal. Chem.* 54:2362–2368 (1982).
42. K. L. Rinehart, Jr., J. C. Cook, Jr., H. Meng, K. L. Olson, and R. C. Pandey. *Nature* 269:832–833 (1977).
43. H. R. Morris, M. Panico, M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler. *Biochem. Biophys. Res. Commun.* 101:623–631 (1981).
44. K. L. Busch, S. E. Unger, A. Vincze, R. G. Cooks, and T. Keough. *J. Am. Chem. Soc.* 104:1507–1511 (1982).
45. C. Fenselau, J. Yergey, and D. Heller. *Int. J. Mass Spectrom. Ion Phys.* 53:5–20 (1983).
46. D. A. Kidwell, M. M. Ross, and R. J. Colton. *J. Am. Chem. Soc.* 106:2219–2220 (1984).
47. (a) K. L. Clay, D. O. Stene, and R. C. Murphy. *Biomed. Mass Spectrom.* 11:47–49 (1984); (b) C. F. Beckner and R. M. Caprioli. *Biomed. Mass Spectrom.* 11:60–62 (1984).
48. J. Franks. *Int. J. Mass Spectrom. Ion Phys.* 46:343–346 (1983).
49. W. V. Ligon, Jr. *Int. J. Mass Spectrom. Ion Phys.* 41:205–208 (1982).
50. D. F. Hunt, W. M. Bone, J. Shabonowitz, J. Rhodes, and J. M. Ballard. *Anal. Chem.* 53:1704–1706 (1981).
51. M. A. Rudat and C. N. McEwen. *Int. J. Mass Spectrom. Ion Phys.* 46:351–354 (1983).
52. W. Aberth, K. M. Straub, and A. L. Burlingame. *Anal. Chem.* 54:2029–2034 (1982).
53. R. Stoll, U. Schade, F. W. Rollgen, U. Giessman, and D. F. Barofsky. *Int. J. Mass Spectrom. Ion Phys.* 43:227–229 (1982).
54. D. F. Barofsky, U. Giessmann, L. W. Swanson, and A. E. Bell. *Int. J. Mass Spectrom. Ion Phys.* 46:495–497 (1983).
55. C. J. McNeal. *Anal. Chem.* 54:43A–50A (1982).
56. C. W. Magee. *Int. J. Mass Spectrom. Ion Phys.* 49:211–221 (1983).
57. A. Van Langenhove, C. E. Costello, H.-F. Chen, J. E. Biller, and K. Biemann. Abstr., 30th Annu. Conf. Mass Spectrom. Allied Topics, Honolulu, Hawaii, June 6–11, 1982, pp. 558–559.
58. K. L. Rinehart, Jr., L. A. Gaudioso, M. L. Moore, R. C. Pandey, J. C. Cook, Jr., M. Barber, R. D. Sedgwick, R. S. Bordoli, and A. N. Tyler. *J. Am. Chem. Soc.* 103:6517–6520 (1981).
59. J. M. Gilliam, P. W. Landis, and J. L. Occolowitz. *Anal. Chem.* 55:1531–1533 (1983).
60. J. C. Cook and B. N. Green. Abstr., 32nd Annu. Conf. Mass Spectrom. Allied Topics, San Antonio, Tex., May 27–June 1, 1984, p. 835.
61. K. L. Rinehart, Jr. *Science* 218:254–260 (1982).
62. K. L. Clay and R. C. Murphy. *Int. J. Mass Spectrom. Ion Phys.* 53:327–330 (1983).
63. W. Rapp and M. Hohn. In A. Benninghoven (ed.), *Ion Formation from Organic Solids*, Springer-Verlag, New York, 1983, pp. 182–187.
64. R. P. Morgan and M. L. Reed. *Org. Mass Spectrom.* 17:537 (1982).
65. S. E. Unger. *Anal. Chem.* 56:363–368 (1984).
66. I. J. Amster and F. W. McLafferty. *Anal. Chem.* 57:1208–1210 (1985).
67. P. A. Lyon, F. W. Crow, K. B. Tomer, and M. L. Gross. *Anal. Chem.* 56:2278–2284 (1984).
68. P. A. Lyon, W. L. Stebbings, F. W. Crow, K. B. Tomer, D. L. Lippstreu, and M. L. Gross. *Anal. Chem.* 56:8–13 (1984).
69. K. B. Tomer, F. W. Crow, H. W. Knoche, and M. L. Gross. *Anal. Chem.* 55:1033–1036 (1983).
70. M. Barber, R. S. Bordoli, R. D. Sedgwick, and L. W. Tetler. *Org. Mass Spectrom.* 16:256–260 (1981).
71. I. Katakuse and D. M. Desiderio. *Int. J. Mass Spectrom. Ion Phys.* 54:1–15 (1983).
72. S. A. Carr, V. N. Reinhold, B. N. Green, and J. R. Hass. *Biomed. Mass Spectrom.* 12:288–295 (1985).
73. N. Neri, G. Sindona, and N. Uccella. *Gazz. Chim. Ital.* 113:197–202 (1983).
74. M. Panico, G. Sindona, and N. Uccella. *J. Am. Chem. Soc.* 105:5607–5610 (1983).

75. E. E. Kingston, J. H. Beynon, and R. P. Newton. *Biomed. Mass Spectrom.* **11**:367–374 (1984).
76. F. W. Crow, K. B. Tomer, M. L. Gross, J. A. McCloskey, and D. Bergstrom. *Anal. Biochem.* 139:243–262 (1984).
77. F. W. Crow, K. B. Tomer, and M. L. Gross. *Mass Spectrom. Rev.* 2:47–76 (1983).
78. I. A. S. Lewis and P. W. Brooks. Abstr., 31st Annu. Conf. Mass Spectrom. Allied Topics, Boston, Mass., May 8–13, 1983, pp. 850–851.
79. P. Dobberstein, E. Korte, G. Meyerhoff, and R. Pesch. *Int. J. Mass Spectrom. Ion Phys.* 46:185–188 (1983).
80. J. G. Stroh, J. C. Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, and K. L. Rinehart. *Anal. Chem.* 57:985–991 (1985).
81. J. G. Stroh, J. C. Cook, K. L. Rinehart, Jr., T. Kihara, Z. Huang, and I. A. S. Lewis. Abstr., 33 Annu. Conf. Mass Spectrom. Allied Topics, San Diego, Calif., May 26–31, 1985, pp. 784–785.
82. Y. Ito, T. Takeuchi, D. Ishii, M. Goto, and T. Mizuno. *J. Chromatogr.* 358:201–207 (1986).
83. M. Barber, R. S. Bordoli, and R. D. Sedgwick. In H. R. Morris (ed.), *Soft Ionization Biological Mass Spectrometry*, Heyden, London, pp. 137–152.
84. K. L. Rinehart, Jr. *Trends Anal. Chem.* 2:10–14 (1983).
85. D. F. Hunt. *Int. J. Mass Spectrom. Ion Phys.* 45:111–123 (1982).
86. C. Fenselau, R. H. Cotter, D. Heller, and J. Yergey. *J. Chromatogr.* 271:3–12 (1983).
87. H. R. Morris. *Int. J. Mass Spectrom. Ion Phys.* 45:331–342 (1982).
88. M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, G. V. Garner, D. B. Gordon, L. W. Tetler, and R. C. Hider. *Biomed. Mass Spectrom.* 9:265–268 (1982).
89. M. Barber, R. S. Bordoli, G. J. Elliott, R. D. Sedgwick, A. N. Tyler, and B. N. Green. *J. Chem. Soc. Chem. Commun.* 936–938 (1982).
90. A. Dell and G. R. Morris. *Biochem. Biophys. Res. Commun.* **106**:1456–1462 (1982).
91. M. Barber, R. S. Bordoli, G. J. Elliott, A. N. Tyler, J. C. Bill, and B. N. Green. *Biomed. Mass Spectrom.* **11**:182–186 (1984).
92. M. Barber, R. S. Bordoli, G. J. Elliott, N. J. Horoch, and B. N. Green. *Biochem. Biophys. Res. Commun.* **110**:753–757 (1983).
93. J. S. Cottrell and B. H. Frank. *Biochem. Biophys. Res. Commun.* 127:1032–1038 (1985).
94. D. H. Williams, C. V. Bradley, S. Santikarn, and G. Bojesen. *Biochem. J.* **201**:105–117 (1982).
95. K. Biemann. *Koenshu-Iyo Masu Kenkyukai* 6:21–32 (1981).
96. M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, and B. W. Bycroft. *Biochem. Biophys. Res. Commun.* **101**:632–638 (1981).
97. G. D. Roberts, S. A. Carr, S. Rottschaefer, and P. W. Jeffs. *J. Antibiot.* 38:713–720 (1985).
98. A. Dell, H. R. Morris, H. Egge, H. von Nicolai, and G. Strecker. *Carbohydr. Res.* **115**:41–52 (1983).
99. K. Harada, M. Suzuki, N. Takeda, A. Tatematsu, and H. Kambara. *J. Antibiot.* 35:102–105 (1982).
100. T. Komori, H. Nanri, Y. Itakura, K. Sakamoto, S. Taguchi, R. Higuchi, T. Kawasaki, and T. Higuchi. *Liebigs Ann. Chem.* 37–55 (1983).
101. N. Saito, C. F. Timberlake, O. G. Tucknott, and I. A. S. Lewis. *Phytochemistry* 22:1007–1009 (1983).
102. I. Jardine, G. F. Scanlan, V. R. Mattox, and R. Kumar. *Biomed. Mass Spectrom.* **11**:4–9 (1984).
103. R. E. Isaac, N. P. Milner, and H. H. Rees. *Biochem. J.* 213:261–265 (1983).
104. H. E. May and D. M. Desiderio. *J. Chem. Soc. Chem. Commun.* 72–73 (1983).
105. G. R. Fenwick, J. Eagles, and R. Self. *Biomed. Mass Spectrom.* **10**:382–386 (1983).
106. S. Takahashi, K. Serita, M. Arai, H. Seto, K. Furihata, and N. Otake. *Tetrahedron Lett.* 24:499–502 (1983).
107. R. K. Mitchum, F. E. Evans, J. P. Freeman, and D. Roach. *Int. J. Mass Spectrom. Ion Phys.* 46:383–386 (1983).
108. L. Grotjahn, R. Frank, and H. Blocker. *Nucl. Acids Res.* **10**:4671–4678 (1982).
109. S. J. Gaskell, B. G. Brownsey, P. W. Brooks, and B. N. Green. *Biomed. Mass Spectrom.* **10**:215–219 (1983).
110. M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, B. N. Green, V. C. Parr, and J. L. Gower. *Biomed. Mass Spectrom.* 9:11–17 (1982).
111. A. I. Cohen, P. T. Finke, and B. N. Green. *J. Pharm. Sci.* 71:1065–1066 (1982).
112. R. C. Tweit, R. C. Pandey, and K. L. Rinehart, Jr. *J. Antibiot.* 35:997–1012 (1982).
113. M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler. *Biomed. Mass Spectrom.* 8:492–495 (1981).
114. A. Dell, R. C. Hider, M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, and J. B. Neilands. *Biomed. Mass Spectrom.* 9:158–161 (1982).
115. A. I. Cohen, K. A. Glavan, and J. F. Kronauge. *Biomed. Mass Spectrom.* **10**:287–291 (1983).
116. R. D. Grigsby, S. E. Scheppele, Q. G. Grindstaff, G. P. Sturm, Jr., L. C. E. Taylor, H. Tudge, C. Wakefield, and S. Evans. *Anal. Chem.* 54:1108–1113 (1982).
117. M. M. Ross and R. J. Colton. *Anal. Chem.* 55:1170–1171 (1983).
118. J. J. Monaghan, M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler. *Org. Mass Spectrom.* 17:569–574 (1982).
119. D. N. Heller, J. Yergey, and R. J. Cotterd. *Ana. Chem.* 55:1310–1313 (1983).
120. K. D. Cook and K. W. S. Chan. *Int. J. Mass Spectrom. Ion Phys.* 54:135–149 (1983).
121. C. Javanaud and J. Eagles. *Org. Mass Spectrom.* 18:93–98 (1983).
122. G. Pelzer, E. DePauw, D. Viet Dung, and J. Marien. *J. Phys. Chem.* 88:5065–5068 (1984).
123. R. G. Finke, M. W. Droege, J. C. Cook, and K. S. Suslick. *J. Am. Chem. Soc.* **106**:5750–5751 (1984).
124. K. S. Suslick, J. C. Cook, B. Rapko, M. W. Droege, and R. G. Finke. *Inorg. Chem.* 25:241–243 (1986).
125. (a) A. L. Burlingame, T. A. Baillie, and P. J. Derrick. *Anal. Chem.* 58:165R–211R (1986); (b) A. L. Burlingame, J. O. Whitney, and D. H. Russell. *Anal. Chem.* 56:417R–467R (1984); (c) A. L. Burlingame, A. Dell, and D. H. Russell. *Anal. Chem.* 54:363R–409R (1982).
126. P. Roepstorff, P. Højrup, and J. Møller. *Biomed. Mass Spectrom.* 12:181–189 (1985).
127. P. Roepstorff and J. Fohlman. *Biomed. Mass Spectrom.* **11**:601 (1984).
128. (a) K. L. Rinehart, Jr., J. C. Cook, Jr., R. C. Pandey, L. A. Gaudioso, H. Meng, M. L. Moore, J. B. Gloer, G. R. Wilson, R. E. Gutowsky, P. D. Zierath, L. S. Shield, L. H. Li, H. E. Renis, J. P. McGovren, and P. G. Canonico. *Pure Appl. Chem.* 54:2409–2424 (1982); (b) K. Biemann. *Int. J. Mass Spectrom. Ion Phys.* 45:183–194 (1982).
129. (a) T. D. Lee, K. Legesse, D. H. Hawke, J. E. Shively, B. S. Rothman, and E. Mayeri. *Biochem. Biophys. Res. Commun.* 132:520–525 (1985); (b) A. Bateman, A. Dell, and H. R. Morris. *J. Appl. Biochem.* 7:126–132 (1985); (c) R. J. Cotter, B. S. Larsen, D. N. Heller, J. E. Campana, and C. Fenselau. *Anal. Chem.* 57:1479–1480 (1985).
130. J. Yergey, D. Heller, G. Hansen, R. J. Cotter, and C. Fenselau. *Anal. Chem.* 55:353–356 (1983).
131. D. Renner and G. Spiteller. *Angew. Chem. Int. Ed. Engl.* 24:408–409 (1985).
132. Y. Wada, A. Hayashi, I. Katakuse, T. Matsuo, and H. Matsuda. *Biomed. Mass Spectrom.* 12:122–126 (1985).
133. A. M. Buko and B. A. Fraser. *Biomed. Mass Spectrom.* 12:577–585 (1985).
134. B. S. Larsen, J. A. Yergey, and R. J. Cotter. *Biomed. Mass Spectrom.* 12:586–587 (1985).
135. H. R. Morris and P. Pucci. *Biochem. Biophys. Res. Commun.* **126**:1122–1128 (1985).
136. (a) R. Self and A. Parente. *Biomed. Mass Spectrom.* 10:78–82 (1983); (b) R. M. Caprioli and T. Fan. *Anal. Biochem.* 154:596–603 (1986).
137. S. L. Murawski, J. C. Cook, and K. L. Rinehart, Jr. Abstr., 33rd Annu. Conf. Mass Spectrom. Allied Topics, San Diego, Calif., May 26–31, 1985, pp. 506–507.
138. J. L. Witten, M. H. Schaffer, M. O'Shea, J. C. Cook, M. E.

Hemling, and K. L. Rinehart, Jr. *Biochem. Biophys. Res. Commun.* **124**:350–358 (1984).
139. G. Gade, G. J. Goldsworthy, M. H. Schaffer, J. C. Cook, K. L. Rinehart, Jr. *Biochem. Biophys. Res. Commun.* **134**:723–740 (1986).
140. R. Ziegler, K. Eckart, H. Schwarz, and R. Keller. *Biochem. Biophys. Res. Commun.* **133**:337–342 (1986).
141. (a) J. M. Wasylyk, J. E. Biskupiak, C. E. Costello, and C. M. Ireland. *J. Org. Chem.* 48:4445–4449 (1983); (b) D. F. Sesin, S. J. Gaskell, and C. M. Ireland. *Bull. Soc. Chim. Belg.* **95**:853–867 (1986).
142. S. Seki, H. Kambara, and H. Naoki. *Org. Mass Spectrom.* **20**:18–24 (1985).
143. J. E. Fitton, D. F. Hunt, J. Marasco, J. Shabanowitz, S. Winston, and A. Dell. *FEBS Lett.* **169**:25–29 (1984).
144. (a) H. R. Morris, M. Panico, G. W. Taylor. *Biochem. Biophys. Res. Commun.* **117**:299–305 (1983); (b) Y. Wada, A. Hayashi, F. Masanori, I. Katakuse, T. Ichihara, H. Nakabushi, T. Matsuo, T. Sakuri, and H. Matsuda. *Biochim. Biophys. Acta* **749**:244–248 (1983); (c) P. Pussi, C. Carestia, G. Fioretti, A. M. Mastrobuoni, and L. Pagano. *Biochem. Biophys. Res. Commun.* **130**:84–90 (1985).
145. M. R. Clench, G. V. Garner, D. B. Gordon, and M. Barber. *Biomed. Mass Spectrom.* **12**:355–357 (1985).
146. B. W. Gibson and K. Biemann. *Proc. Natl. Acad. Sci. USA* **81**:1956–1960 (1984).
147. T. A. Webster, B. W. Gibson, T. Keng, K. Biemann, and P. Schimmel. *J. Biol. Chem.* **258**:10637–10641 (1983).
148. A. C. Dianoux, A. Tsugita, and M. Przybylski. *FEBS Lett.* **174**:151–156 (1984).
149. B. W. Gibson, W. C. Herlihy, T. S. A. Samy, K.-S. Hahm, H. Maeda, J. Meienhofer, and K. Biemann. *J. Biol. Chem.* **259**:10801–10806 (1984).
150. T. Takao, H. Watanabe, and Y. Shimonishi. *Eur. J. Biochem.* **146**:503–508 (1985).
151. T. Takao, T. Hitouji, Y. Shimonishi, T. Tanabe, S. Inouye, and M. Inouye. *J. Biol. Chem.* **259**:6105–6109 (1984).
152. K. Fukuhara, T. Tsuji, K. Toi, T. Takao, and Y. Shimonishi. *J. Biol. Chem.* **260**:10487–10494 (1985).
153. R. J. Robb, R. M. Kutny, M. Panico, H. R. Morris, and V. Chowdhry. *Proc. Natl. Acad. Sci. USA* **81**:6486–6490 (1984).
154. K. Eckart, H. Schwarz, K. B. Tomer, and M. L. Gross. *J. Am. Chem. Soc.* **107**:6765–6769 (1985).
155. J. G. Stroh, K. L. Rinehart, Jr., J. C. Cook, T. Kihara, M. Suzuki, and T. Arai. *J. Am. Chem. Soc.* **108**:858–859 (1986).