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High-resolution laser desorption mass spectrometry of peptides and small proteins

(matrix-assisted laser desorption/insulin/melittin/vasopressin/isotopes)

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ABSTRACT **Matrix-assisted laser desorption/ionization (MALDI) has been used with an external ion source Fourier-transform mass spectrometer to obtain the highest mass resolution ever, to our knowledge, demonstrated for laser-produced ions ($m/\Delta m$ = 1,100,000 for [Arg^8]vasopressin, 228,000 for melittin, and 90,000 for bovine insulin). The peaks in the isotope cluster for bovine insulin are fully resolved, and the mass measurement accuracy is an order of magnitude better than can be achieved with time-of-flight mass spectrometry. With the method described here, analyte is applied to a sample probe and mixed with a solution containing a matrix material (2,5-dihydroxybenzoic acid) that strongly absorbs ultraviolet light. Upon irradiation with a pulse from an excimer laser (353 nm, 2 mJ), a large number of intact protonated molecular ions are produced. The ions are focused by a 117-cm-long quadrupole ion guide and injected into an ion cyclotron resonance analyzer cell located inside the bore of a 6.5-T superconducting magnet. A pulse of argon buffer gas cools the ions prior to detection. One of the principal advantages of an external ion source Fourier-transform mass spectrometer is that the ion formation and ion detection processes are separated and can be independently optimized.**

In 1988, Karas and Hillenkamp introduced a mass spectrometry method called matrix-assisted laser desorption/ionization (MALDI) for measuring the molecular weights of biopolymers (1–3). The key idea is to isolate and surround analyte biomolecules with a matrix material that strongly absorbs laser light. When an incident laser pulse strikes the solid matrix/biomolecule mixture, ablation and ionization of the surface layer occurs very rapidly. One might expect that fragile biomolecules would not survive such a violent event, but, remarkably, they remain intact and appear as the main peaks in a mass spectrum. Selection of an appropriate matrix material has proven to be critical to the success of MALDI. Beavis and Chait screened hundreds of possible matrices and found that cinnamic acid derivatives are excellent for the analysis of peptides and proteins (4, 5). With the introduction a few years ago of inexpensive MALDI mass spectrometers, the technique has become an essential tool in many protein biochemistry laboratories (6, 7).

Most MALDI applications use time-of-flight mass spectrometry (TOF-MS) to mass analyze and detect the laser-produced ions. Although this method is rapid and has picomolar sensitivity, the mass measurement accuracy is somewhat limited. Several processes that limit the mass resolution and mass measurement accuracy of TOF mass spectrometers have been identified (8). One cause of peak broadening is the considerable energy spread of the ions. Even at the threshold for ionization, the energy spread can be as large as 30 eV, and as the laser power is increased the spread increases further (9–11). This causes there to be a distribution of arrival times and a broadening of the peaks in the TOF mass spectrum. Further peak broadening can occur when the ions undergo metastable decay in the flight tube (12). Finally, mass measurement errors are caused by adducts of the analyte with Na^+ or losses of NH_3 and H_2O, which create peaks that cannot be resolved.

When MALDI was introduced, it seemed ideal for use with Fourier-transform mass spectrometry (FTMS) because the solid matrix used in MALDI was compatible with the need of FTMS for very low analyzer pressures. Compared with TOF-MS, there were several potential advantages of FTMS, including higher mass resolution and selected ion storage for mixture analysis and sequencing. The early optimism faded in 1991 when the MALDI-FTMS experiments showed much lower mass resolution than had been anticipated (13–15). Even more serious, however, was the observation that peptides having molecular masses greater than 2500 Da failed to produce useful signals. There appeared to be an upper mass limit for high-resolution FTMS detection of biological molecules. Such a limit was suggested in 1987 by Hunt and coworkers (16). They found that FTMS with a liquid secondary ion mass spectrometry (LSIMS) source gave very high mass resolution for small peptides such as neurotensin (m/z 1674) but strikingly lower mass resolution and lower sensitivity for larger peptides such as melittin (m/z 2847), glucagon, and bovine insulin. Progress was made in 1988 when Ijames and Wilkins (17) demonstrated a mass resolution of 60,000 for laser desorption of poly(propylene glycol) up to m/z 5922. A few years later, McLafferty and coworkers (18, 19) reported high mass resolution for peptides and proteins analyzed in a FTMS instrument with an electrospray ionization (ESI) source. Although these results were very impressive, concerns about an intrinsic upper mass limit for FTMS in detecting biopolymer ions above m/z 2500 persisted because the multiply charged ions made by ESI have a mass-to-charge ratio below 2500.

To overcome the apparent detection limitations of FTMS, modified analyzer cell designs and tailored excitation methods were investigated (20–23). In 1992, Solouki and Russell (24) tested a special FTMS device with an external "waiting room" for collisional relaxation of laser-produced ions. Although they did not succeed in achieving high mass resolution, their experiments demonstrated that high trapping potentials and collisional relaxation were needed to detect high mass ions. The most successful MALDI-FTMS experiments to date have been performed by Wilkins and coworkers (25, 26). By using carefully timed pulses to trap the ions and a sugar comatrix to minimize metastable decay, they obtained a mass resolution ($m/\Delta m$) in excess of 100,000 for small peptides and 27,300 for bovine insulin (26).

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Abbreviations: MALDI, matrix-assisted laser desorption/ionization; TOF, time-of-flight; FTMS, Fourier-transform mass spectrometry; FWHM, full width at half maximum.

In this paper we demonstrate that ions produced by MALDI can be detected at high mass resolution with a specially designed external ion source Fourier-transform mass spectrometer. The same biopolymers that failed previously and were cited as evidence for an "upper mass limit" give excellent-quality high-resolution mass spectra with this method. For example, the mass resolution for bovine insulin is 90,000, and the isotope cluster peaks are readily resolved. This capability is important for accurate measurements of molecular weights and determining if a protein has been deaminated. A key feature of the new method is that ion production and ion detection are separated, thereby making it possible to optimize each process independently.

MATERIALS AND METHODS

The mass spectrometer used in this work is an external ion source Fourier-transform mass spectrometer that was built several years ago at the University of California, Irvine (27–32). Samples prepared for MALDI analysis are deposited on the tip of a direct probe and inserted into the ion source. When the sample is irradiated with a laser pulse, ions are extracted from the source and passed into a long quadrupole ion guide that focuses and transports them to an FTMS analyzer cell located inside the bore of a superconducting magnet.

There are four basic parts to the FTMS instrument: magnet, laser, data system, and vacuum system. The magnet is a 6.5-T horizontal bore (15-cm diameter) superconducting magnet made by Oxford Instruments. The homogeneity is 10 ppm over a 16-cm^3 cubic volume located at the center of the magnet. The laser used for MALDI experiments is an excimer laser (EMG 201MSC, Lambda Physik, Acton, MA) operated with xenon fluoride at 353 nm. Typically 2 mJ per pulse is applied to the sample at a fluence of 500 mJ/cm^2. A small helium–neon laser is aligned collinear with the excimer beam for sighting on the target.

The mass spectrometer is controlled by an Omega 586 data system developed and manufactured by IonSpec Corporation. Two special features are a large buffer memory (1,048,576 words) in the transient digitizer and a 24-channel programmable digital-to-analog converter unit for controlling the voltages on the analyzer cell plates to a time resolution of 1 μs. In FTMS there is always a trade-off between mass range and mass resolution. To achieve high mass resolution, the cyclotron resonance signals produced by the ions must be digitized for several seconds. To avoid overflowing the buffer memory, therefore, signals from the preamplifier are passed through a mixer circuit that down-shifts the frequencies to a range that is more easily digitized. This is called the mixer, heterodyne, or narrowband detection mode. Mass spectra in this paper were acquired with an analog-to-digital converter clock rate of either 10 or 20 kHz, and 524,288 words of data were acquired. A full Hanning apodization window and one-zero fill were used in the magnitude mode Fourier-transform calculations. If the apodization window is not used, the mass resolution (full width at half maximum, FWHM) is higher by about a factor of 2 and the bases of the peaks are considerably broadened.

The vacuum system of the FTMS instrument has three differentially pumped chambers which hold the ion source, the quadrupole ion guide, and the FTMS analyzer cell. Each chamber has its own cryogenic pump (Air Products APD-6; Air Products and Chemicals, Allentown, PA). After bakeout, the base pressure in the analyzer cell region is typically 2×10^{-10} torr (1 torr = 133 Pa). The ions are extracted from the source region and injected into the center of a 117-cm-long quadrupole ion guide. The purpose of the ion guide is to focus the ions and enable them to pass through the intense fringing fields of the superconducting magnet (27, 31). Normally, it is operated in the rf-only mode so that it functions like a bandpass filter with adjustable high mass and low mass cutoffs. For example, when operated at 500 V peak-to-peak, ions between m/z 530 and m/z 6000 are transmitted to the analyzer cell. As the ions exit from the quadrupole ion guide, they are focused by the intense magnetic field and trapped inside the FTMS analyzer cell. The dimensions of the analyzer cell are 5 cm × 5 cm × 7.5 cm, and the six plates of the cell are highly polished 304 stainless steel.

In a typical MALDI experiment, the data system first triggers the opening of a valve (General Valve Corp. series 90; Fairfield, NJ) for 2 ms to admit a pulse of argon gas into the analyzer cell region. At the same time, the quadrupole ion guide is energized and the potentials on the trapping plates of the FTMS analyzer cell are set at 0 V on the front trapping plate and 10 V on the rear trapping plate. After a delay time of 50 ms, the laser is fired once and the ions move rapidly down the quadrupole ion guide. They enter the analyzer cell after about 0.5–0.8 ms, at which time the potential on the front trapping plate is pulsed rapidly to 10 V. The mass range of ions trapped in the analyzer cell depends on two factors. First, the range of ions injected into the analyzer cell depends on the bandpass of the quadrupole ion guide, as described above. Second, there is a TOF effect because the pulse on the trapping plate selects only ions with a particular flight time, plus or minus about 0.1 ms. Since at threshold the MALDI mechanism generates ions with a common speed (9) of about 600 m/s, the arrival times are not as variable as might be expected. These two effects limit the range of masses trapped to typically a few thousand daltons at any one time. Of course, the pulse delay time and quadrupole rf voltage can readily be changed to trap a different range of masses. While the ions are trapped inside the analyzer cell, they undergo multiple collisions with the argon buffer gas and relax to the center of the analyzer cell. After a delay time of typically 50 s, the potentials on the trapping plates are lowered to 0.5 V prior to acceleration by an rf chirp and detection.

Analyte solutions for the MALDI experiments were prepared by dissolving approximately 1 mg of peptide in 1 ml of an acetonitrile/water solution (50/50, vol/vol). The matrix solution was prepared by adding 10 mg of 2,5-dihydroxybenzoic acid to 1 ml of ethanol (33). Addition of D-fructose to the matrix solution was an important factor in suppressing fragmentation (34). [Arg^8]Vasopressin, melittin, B chain of bovine insulin, and bovine insulin were obtained from Sigma, and 2,5-dihydroxybenzoic acid was obtained from Aldrich. All samples were used without further purification. Equal 0.5-μl volumes of the analyte and matrix solutions were delivered to a stainless steel sample probe and allowed to evaporate to dryness prior to insertion into the vacuum lock of the ion source.

RESULTS AND DISCUSSION

Several peptides and small proteins have been analyzed successfully in the external ion source FTMS instrument, using the pulsing scheme described above. Fig. 1*A* shows a high-resolution mass spectrum for [Arg^8]vasopressin. The narrowband detection mode was used with 524,288 data points acquired over the mass range m/z 1080 to 1090. One laser pulse produces a signal-to-noise ratio greater than 2500:1. The base peak at m/z 1084.446 is the "monoisotopic" peak for the intact protonated peptide. It is isotopically pure; i.e., all the carbon atoms in the molecule are ^{12}C, all the nitrogen atoms are ^{14}N, all the oxygen atoms are ^{16}O, etc. The three peaks at higher mass, each separated by 1 Da, result from incorporation of one or more of the less abundant isotopes into the molecule, with ^{13}C at a natural abundance of 1.11% being the main contributor. It is difficult to measure the mass resolution in Fig. 1*A* because the peaks are so sharp,

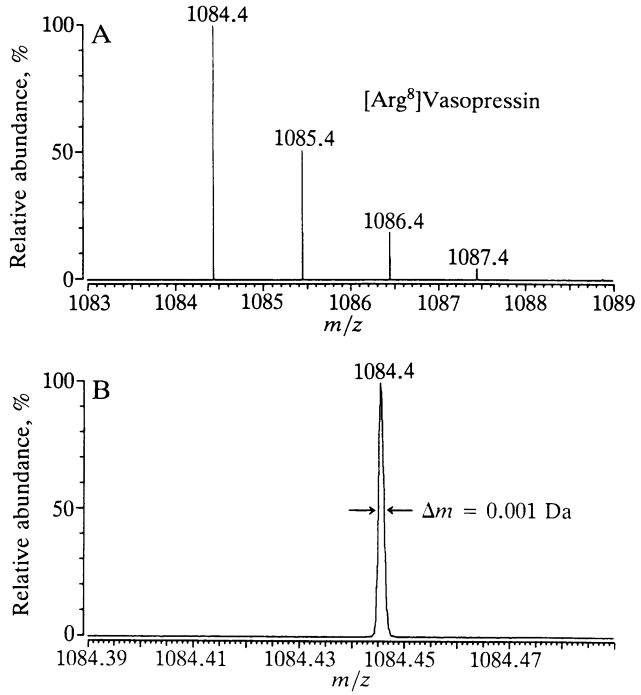

FIG. 1. High-resolution laser desorption mass spectrum for [Arg^8]vasopressin obtained with an external ion source Fourier-transform mass spectrometer. (*A*) Narrowband acquisition from m/z 1080 to 1090, showing the isotopic cluster peaks for the protonated molecular ion. (*B*) Data plotted with an expanded mass axis to show a mass resolution of 1,100,000.

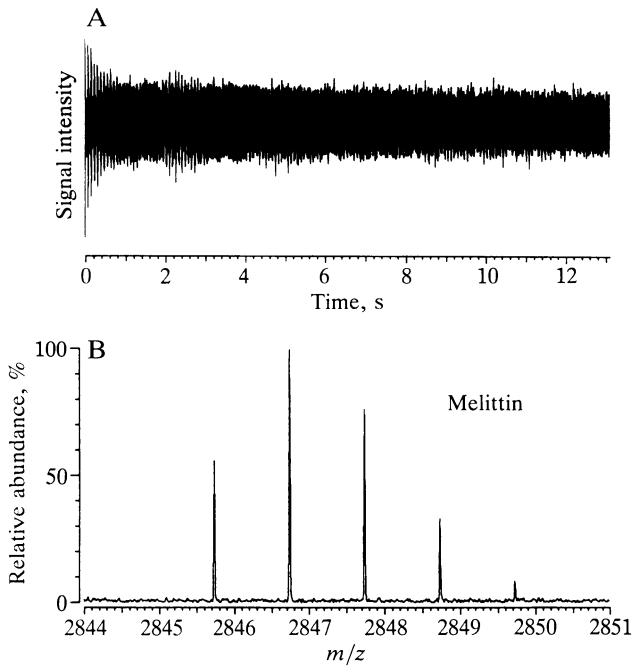

FIG. 2. FTMS data for MALDI of melittin. (*A*) Coherent transient cyclotron resonance signal lasting 13.2 s (one laser pulse, narrowband acquisition at 20 kHz with 262,144 points from m/z 2600 to 3000). (*B*) Resolved isotope cluster for melittin, showing a mass resolution of 228,000 (FWHM).

but the expanded mass scale in Fig. 1*B* shows that the monoisotopic peak is only 0.001 Da wide and the mass resolution $m/\Delta m$ = 1,100,000 (FWHM). This is, to our knowledge, the highest mass resolution ever reported for laser-produced ions in this mass range.

One of the disadvantages of FTMS is the long time scale required for mass analysis. TOF and magnetic sector mass spectrometers accelerate the ions to several kilovolts and detect them after less than a millisecond. In contrast, FTMS requires that the ions remain stable and rotate coherently for several hundred milliseconds, or even several tens of seconds, to get high mass resolution. An approximate formula for mass resolution in FTMS is $m/\Delta m = (f\tau)/2$, where f is the cyclotron frequency of an ion and τ is the time the coherent cyclotron motion is monitored. This equation shows that to achieve unit mass resolution at m/z 5000, the cyclotron resonance signal must last and be digitized for about 0.5 s (with a 6.5-T magnet). Any process that removes ions from the ensemble or interrupts their coherent cyclotron motion will lower the mass resolution.

The effect of metastable decay on FTMS mass resolution is of concern because intense fragmentation of peptides and proteins has been observed in TOF studies of MALDI (12). Although we have seen evidence for metastable decay in FTMS, it does not seem to be a major problem, particularly if a material such as D-fructose is added to the matrix, as suggested by Wilkins *et al.* (34). Evidence for this is provided by Fig. 2*A*, which shows a coherent transient cyclotron resonance signal for melittin lasting 13 s. During the first second after acceleration, there is a 50% drop in the signal, but after that it continues without damping, indicating that the ions are stable and do not undergo metastable decay. Calculating the Fourier transform of the transient in Fig. 2*A* gives the mass spectrum in Fig. 2*B*, where the resolution is 228,000 for the protonated intact molecular ions of melittin. Melittin is one of the compounds that failed to give high mass resolution in the earlier experiments of Hunt *et al.* (16) and it was cited as an example of how FTMS failed to give high mass resolution. Recently, however, Castoro and Wilkins (26) were also successful in obtaining high mass resolution for melittin. They acquired a transient signal lasting 3.28 s and reported a mass resolution of 99,300 (FWHM). In their analysis of the data, an apodization window was not used in the Fourier-transform calculation. If we omit the apodization window from our analysis, the transient in Fig. 2 gives a mass resolution of 376,000. These results are consistent with the mass resolution equation given above, because in our experiment the transient was digitized for 13 s, or about 4 times longer than the signal acquired by Castoro and Wilkins.

External ion source FTMS-MALDI data for the B chain of bovine insulin are shown in Fig. 3*A*. In this case, the mass resolution is 186,000 for the isotopic cluster of the intact protonated molecular ion. One laser pulse was used, and the narrowband acquisition was from m/z 3300 to 3700. The matrix was 1:1 2,5-dihydroxybenzoic acid with D-fructose as a comatrix. In the past, one of the problems with FTMS has been that it gives unreliable isotope ratios, especially when space charge effects resulting from too many ions in the analyzer cell cause the cyclotron resonance signals to be perturbed. With the new Omega 586 electronics, however, this problem has been overcome by using a more sensitive preamplifier that can detect weak cyclotron resonance signals produced by ion concentrations well below the space charge limit. This improvement is demonstrated by the excellent agreement between the experimental (Fig. 3*A*) and theoretical (Fig. 3*B*) isotope ratios for the B chain of bovine insulin. The excellent signal-to-noise ratio and high mass resolution in Fig. 3*A* demonstrate convincingly that there are no intrinsic limitations of FTMS for detecting high mass biopolymers.

Fig. 4 shows an external ion source FTMS-MALDI mass spectrum of bovine insulin. The matrix was a 1:1 mixture of 2,5-dihydroxybenzoic acid and D-fructose. The signal-to-noise ratio is about 50:1 for one laser pulse. The isotope cluster for the intact protonated molecular ion of bovine

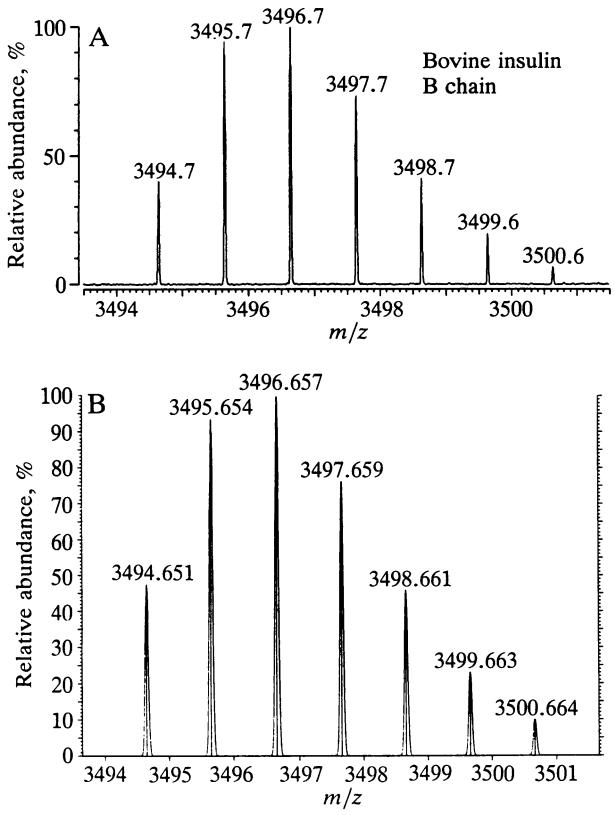

FIG. 3. External ion source FTMS-MALDI data for the B chain of bovine insulin. (*A*) Mass resolution of 186,000 for the isotope cluster of the protonated molecular ion. (*B*) Calculated isotope distribution for the protonated molecular ion of bovine insulin B chain, $C_{157}H_{233}N_{40}O_{47}S_2$.

insulin is clearly seen at a mass resolution of 90,000 (FWHM), and a second cluster of peaks, 17 Da below the protonated parent, results from loss of NH_3.

In the experiments reported above, we have not used the "ion axialization" and "time-base correction" methods that have been proposed by others for improving FTMS mass resolution. With ion axialization, the axial and cyclotron motions of the ions are cooled by a buffer gas, and the outward radial diffusion caused by the buffer gas is counteracted by an azimuthal quadrupole rf field (35–39). With time-base correction, time-dependent shifts in the cyclotron frequency are measured for a particular peak in the mass spectrum, the shifts are fit to a fourth-order equation, and the original experimental data are corrected to eliminate the shift problem (40). Using these methods, Guan and Marshall (41)

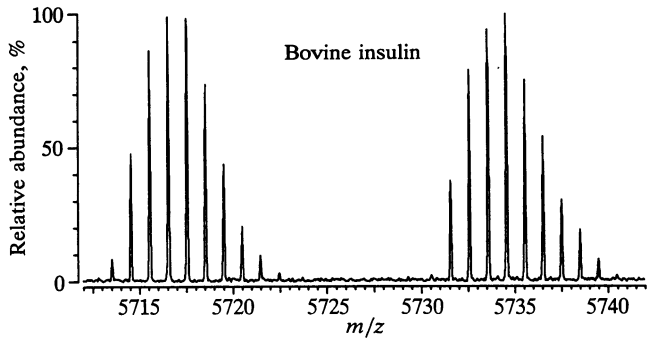

FIG. 4. High-resolution ($m/\Delta m$ = 90,000 FWHM) laser desorption mass spectrum of bovine insulin, showing a cluster of peaks for the protonated molecular ion and loss of ammonia from the protonated molecular ion.

demonstrated that dampened and frequency-shifted data for the $[M + K]^+$ adduct of leucine enkephalin, m/z 594, could be transformed from an original mass resolution of about 30,000 to 1,700,000. These methods certainly work in the test cases that have been tried, but it remains to be seen whether they will be generally useful and practical. We expect that the mass resolution performance achieved presently with our external ion source FTMS method (90,000 for bovine insulin) will be sufficient for most practical applications and that the ion axialization and time-base correction schemes will not be needed.

In conclusion, we are very encouraged by these initial studies. It is clear that MALDI can be very effectively mated with an external ion source, quadrupole ion guide Fourier-transform mass spectrometer to achieve very high mass resolution and picomolar sensitivity. We hope that these results will put to rest the concerns about an intrinsic upper mass limit for FTMS in detecting high mass biological ions. One of the advantages of the external ion source method is that the conditions for efficient laser desorption can be optimized independently of the conditions for efficient high-resolution ion detection in the FTMS analyzer cell. This is difficult to do in MALDI-TOF experiments because a change in the laser power or the source extraction potentials alters the flight time and the mass resolution. It may be possible to extend these methods to molecules larger than bovine insulin. We are also interested in developing the selective ion storage capabilities of FTMS so that mixtures of peptides can be analyzed and sequenced by photodissociation or collisional dissociation.

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1. Karas, M. & Hillenkamp, F. (1988) *Anal. Chem.* **60**, 2291–2301.
2. Karas, M., Bahr, U. & Hillenkamp, F. (1989) *Int. J. Mass Spectrom. Ion Processes* **92**, 231–242.
3. Hillenkamp, F., Karas, M., Beavis, R. C. & Chait, B. T. (1991) *Anal. Chem.* **63**, 1193 (abstr.).
4. Beavis, R. C. & Chait, B. T. (1989) *Rapid Commun. Mass Spectrom.* **3**, 432–435.
5. Beavis, R. C., Chaudhary, T. & Chait, B. T. (1992) *Org. Mass Spectrom.* **27**, 156–158.
6. Billeci, T. M. & Stults, J. T. (1993) *Anal. Chem.* **65**, 1709–1716.
7. Chait, B. T. & Kent, S. B. H. (1992) *Science* **257**, 1885–1894.
8. Cotter, R. C. (1992) *Anal. Chem.* **64**, 1027 (abstr.).
9. Beavis, R. C. & Chait, B. T. (1991) *Chem. Phys. Lett.* **181**, 479–484.
10. Zhou, J., Ens, W., Standing, K. C. & Verentchikov, A. (1992) *Rapid Commun. Mass Spectrom.* **6**, 671–678.
11. Pan, Y. & Cotter, R. J. (1992) *Org. Mass Spectrom.* **27**, 3–8.
12. Spengler, B., Kirsch, D. & Kaufmann, R. (1991) *Rapid Commun. Mass Spectrom.* **5**, 198–202.
13. Hettich, R. L. & Buchanan, M. V. (1991) *J. Am. Soc. Mass Spectrom.* **2**, 22–28.
14. Hettich, R. L. & Buchanan, M. V. (1991) *J. Am. Soc. Mass Spectrom.* **2**, 403–412.
15. Hettich, R. L. & Buchanan, M. V. (1991) *Int. J. Mass Spectrom. Ion Processes* **111**, 365–380.
16. Hunt, D. F., Shabanowitz, J., Yates, J. R., III, Zhu, N.-Z., Russell, D. H. & Castro, M. E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 620–623.
17. Ijames, C. & Wilkins, C. L. (1988) *J. Am. Chem. Soc.* **110**, 2687–2688.
18. Henry, K. D., Quinn, J. P. & McLafferty, F. W. (1991) *J. Am. Chem. Soc.* **113**, 5447–5449.
19. Beu, S. C., Senko, M. W., Quinn, J. P., Wampler, F. M., III & McLafferty, F. W. (1993) *J. Am. Soc. Mass Spectrom.* **4**, 557–565.
20. Wang, M. & Marshall, A. G. (1990) *Anal. Chem.* **62**, 515–520.
21. Hanson, C. D., Castro, M. E., Kerley, E. L. & Russell, D. A. (1990) *Anal. Chem.* **62**, 520–526.

22. Caravatti, P. & Allemann, M. (1991) *Org. Mass Spectrom.* **26,** 514–518.
23. Beu, S. C. & Laude, D. A., Jr. (1992) *Int. J. Mass Spectrom. Ion Processes* **112,** 215–230.
24. Solouki, T. & Russell, D. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5701–5704.
25. Castoro, J. A., Koster, C. & Wilkins, C. L. (1992) *Rapid Commun. Mass Spectrom.* **6,** 239–241.
26. Castoro, J. A. & Wilkins, C. L. (1993) *Anal. Chem.* **65,** 2621–2627.
27. McIver, R. T., Jr. (1985) U.S. Patent 4,535,235.
28. McIver, R. T., Jr., Hunter, R. L. & Bowers, W. D. (1985) *Int. J. Mass Spectrom. Ion Processes* **64,** 67–77.
29. Lebrilla, C. B., Amster, I. J. & McIver, R. T., Jr. (1989) *Int. J. Mass Spectrom. Ion Processes* **87,** R7–R13.
30. Lebrilla, C. B., Wang, D. T.-S., Mizoguchi, T. J. & McIver, R. T., Jr. (1989) *J. Am. Chem. Soc.* **111,** 8593–8598.
31. McIver, R. T., Jr. (1990) *Int. J. Mass Spectrom. Ion Processes* **98,** 35–50.
32. Lebrilla, C. B., Wang, D. T.-S., Hunter, R. L. & McIver, R. T., Jr. (1990) *Anal. Chem.* **62,** 878–880.
33. Strupat, K., Karas, M. & Hillenkamp, F. (1991) *Int. J. Mass Spectrom. Ion Processes* **111,** 89–102.
34. Koster, C., Castoro, J. A. & Wilkins, C. L. (1992) *J. Am. Chem. Soc.* **114,** 7572–7574.
35. Wineland, D. J. & Dehmelt, H. G. (1975) *Int. J. Mass Spectrom. Ion Phys.* **16,** 338–342.
36. Wineland, D. J. & Dehmelt, H. G. (1976) *Int. J. Mass Spectrom. Ion Phys.* **19,** 251–255.
37. Savard, G., Becker, S., Bollen, G., Dluge, H.-J., Moore, R. B., Otto, T., Schweikhard, L., Stolzenberg, H. & Wiess, U. (1991) *Phys. Lett. A* **158,** 247–252.
38. Schweikhard, L., Guan, S. & Marshall, A. G. (1992) *Int. J. Mass Spectrom. Ion Processes* **120,** 71–83.
39. Speir, J. P., Gorman, G. S., Pitsenberger, C. C., Turner, C. A., Wang, P. P. & Amster, I. J. (1993) *Anal. Chem.* **65,** 1746–1752.
40. Bruce, J. E., Anderson, G. A., Hofstadler, S. A., Winger, B. E. & Smith, R. P. (1993) *Rapid Commun. Mass Spectrom.* **7,** 700–703.
41. Guan, S. & Marshall, A. G. (1993) *Rapid Commun. Mass Spectrom.* **7,** 857–860.