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Laser desorption studies of high mass biomolecules in Fourier-transform ion cyclotron resonance mass spectrometry

(insulin/nitroaniline/matrix-assisted laser desorption/kinetic energy)

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ABSTRACT **Matrix-assisted laser desorption ionization is used to obtain Fourier-transform ion cyclotron resonance mass spectra of model peptides (e.g., gramicidin S, angiotensin I, renin substrate, melittin, and bovine insulin). Matrix-assisted laser desorption ionization yields ions having appreciable kinetic energies. Two methods for trapping the high kinetic energy ions are described: (*i*) the ion signal for $[M + H]^+$ ions is shown to increase with increasing trapping voltages, and (*ii*) collisional relaxation is used for the detection of $[M + H]^+$ ions of bovine insulin.**

Ionization methods such as californium plasma desorption (1), matrix-assisted laser desorption (MALD) (2), and electrospray ionization (ESI) (3) have greatly expanded the role of mass spectrometry of high mass biomolecules. Biomolecules having molecular masses >100,000 Da can be ionized to yield a single-charged (e.g., $[M + H]^+$) or multiple-charged (e.g., $[M + nH]^{n+}$) ion. Although most of the MALD and ESI work has utilized time-of-flight, quadrupole mass spectrometers and/or quadrupole ion traps, MALD (4) and ESI are adaptable to Fourier-transform ion cyclotron resonance (FT-ICR). The advantage of FTICR (and the quadrupole ion trap) is that tandem mass spectrometry experiments [e.g., collision-induced dissociation and photodissociation (5)] and ion molecule reactions can be implemented for structural characterization. The best high mass performance [≈60,000 mass resolution at m/z 6000 (6)] of FTICR has been achieved with laser desorption ionization of nonpolar polymers. McLafferty and coworkers (7) have made considerable progress with the development of ESI for FTICR, and a high-sensitivity ESI/FTICR system was recently described.* Several groups (9, 10) have suggested that the performance of FTICR with biological molecules (samples that have relatively low ionization probabilities) and molecular masses greater than ≈2500 Da may be adversely affected by inefficient trapping of the ions or inherent limitations of the ion detection. For example, the mass resolution for samples with molecular masses greater than ≈2500 Da is far less than the theoretically predicted values (11). On the basis of our experience with desorption ionization and high mass FTICR, we suggested that the major problem with high-resolution FTICR at high mass is due to the high kinetic energies of desorbed ions (9). We have recently evaluated two experimental methods for improving the trapping efficiency of high mass ions: (*i*) the effects of increasing the electric trapping field and (*ii*) the effects of collisional relaxation of the laser-desorbed species prior to trapping the ions in the ion cyclotron resonance (ICR) cell. In this article we present the results from both experimental methods. The results show that increasing the trapping potentials improves the ion trapping efficiency, but detection of $[M + H]^+$ ions of bovine insulin (m/z = 5734) requires collisional relaxation of ions prior to their introduction into the ion cell.

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MATERIALS AND METHODS

FTICR Mass Spectrometer. The FTICR mass spectrometer used in this study consists of a home-built ICR cell and a vacuum system. The mass spectrometer is equipped with a 7.0-T superconducting Oxford magnet and Extrel FTMS-2001 data acquisition system. A Laser Science cartridge-type pulsed N_2 laser (model VSL-33ND) is used for ionization. The laser delivers ≈200 μJ of pulse energy at 337.1 nm per 3-ns pulse. The maximum repetition rate of the laser is 20 Hz with intensity stability of 4% at 10 Hz. A 200.0-cm focal length lens mounted outside the vacuum chamber focuses the laser beam through a UV-grade sapphire window to a second focusing lens of 20.0-cm focal length that is mounted onto the ICR cell (Fig. 1). The second lens focuses the collimated laser beam to a spot size approximately 20 μm by 20 μm onto a direct insertion sample probe surface. The laser beam is incident on the target surface in a perpendicular fashion. The desorbed ions are trapped in a 16-cm-long, 4-cm-diameter cylindrical cell made of oxygen-free copper. For typical experiments, the sample probe tip is placed about 5 mm outside the ion cell, and ions are transferred into the cell through an aperture of 0.45 cm in diameter.

MALD ionization mass spectra were obtained at trapping voltages of 1–9 V (6.25 V/m–56.25 V/m). The bias voltage applied to the sample probe was kept constant at 0.0 V. Fig. 2 illustrates the sequence of events for a single laser shot. First, positive and negative ions inside the cell are removed in quench events. The removal of ions is accomplished by first applying +9.75 V to both trap plates for 50 ms (negative ion quench), which is then followed by a 50-ms positive ion quench event where trapping plates are set at −9.75 V. After a delay of 50 μs (D1) a −5 V logic pulse from the FTICR cell controller initiates a single firing of the laser. At the time of the laser pulse, the trapping plates are at ground potential. A second variable delay (D2) permits ions to enter into the cell before the trapping voltage (VTR) is applied to the trap plates. Positive or negative ions can be selectively trapped by applying positive (for positive ions) or negative (for negative ions) voltages to the trap plates. Ion detection is accomplished by chirp excitation with a radiofrequency field. Ion detection is performed at medium resolution (16,000–32,000 data points) in the direct broadband mode.

Abbreviations: VTR, trapping voltage; MALD, matrix-assisted laser desorption; ESI, electrospray ionization; FTICR, Fourier-transform ion cyclotron resonance; ICR, ion cyclotron resonance.

*Laude, D. A., Jr., Riegner, D. & Beu, S. C., Toward Quantitative Ion Dissociation Measurements in the FTICR Cell, Federation of Analytical Chemistry and Spectroscopy Societies and Pacific Conference on Chemistry and Spectroscopy (FACSS XVIII/PCCS XIII), September 1991, Anaheim, CA.

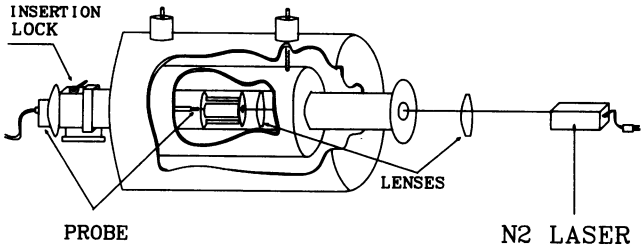

FIG. 1. Diagram of the MALD/FTICR system.

In the pulsed-valve experiments, the gaseous reagents (inert collisional gases such as He and Ar) are admitted into the vacuum system by using a General Valve (model 9-337-900) pulsed valve. The timing of the reagent gas pulse sequence is controlled in such a manner that molecules of the collision gas collide with desorbed species immediately after the laser is fired. "Waiting room" experiments of laser-desorbed ions are accomplished by placing the sample probe inside a brass housing. A close-up view of the probe, trapping cell, and external housing (waiting room) is shown in Fig. 3. The direct insertion sample probe forms a tight seal against the housing, and ions and neutrals formed by the incident laser irradiation escape via a 0.25-cm diameter aperture. After laser irradiation, desorbed species inside the waiting room undergo collisions to lose most of their initial kinetic energies; translationally relaxed ions are transferred into the ICR cell and mass analyzed. The same experimental sequence (as described above) is used for waiting room experiments except that the sample probe bias voltage is set at positive potentials to force the positive ions into the trapping cell. The volume inside the waiting room can be varied by moving the sample probe.

Sample Preparation. All chemicals were obtained from Sigma and were used without any further purification. Analyte samples (gramicidin S, angiotensin I, renin substrate, melittin, and bovine insulin) of 0.1 mg were dissolved in 1 ml of ethanol. The matrix was prepared by dissolving 1 mg of 4-nitroaniline in 1 ml of ethanol. Mixtures (1:1, vol/vol) of analyte and matrix solution (≈1 μl of each) were delivered to the sample probe tip surface. The solvent was allowed to evaporate, leaving a thin film of matrix/analyte on the probe tip.

RESULTS AND DISCUSSION

Previous studies of MALD with FTICR on biomolecules such as oligonucleotides and peptides were successful for only relatively low molecular mass samples (e.g., 1000–2000 Da or less) (10, 12). In part, the limited mass range and mass resolution are due to the volatility of the matrix; matrices such as nicotinic acid and sinapic acid are simply too volatile for the (10^{-8} torr; 1 torr = 133 Pa) vacuum requirement for FTICR. It is also important to consider the effects of nonthermal kinetic energies on the detection of high m/z ions (13). Recent work by Beavis and Chait (14), Pan and Cotter (15), and Standing and coworkers (16) suggests that the ions formed by MALD have relatively large kinetic energies. For example, Beavis and Chait (14) report an average axial velocity of 750 m/s for polypeptide molecular ions (independent of m/z) produced by MALD. A value of 2 eV of axial translational energy for molecular ions of [Arg^8]vasopressin (m/z = 1085) with an energy spread of 8 eV (full width at half-maximum) has been reported.† Measurements of Pan and Cotter (15) also suggest that higher mass ions are desorbed with increased kinetic energies. The initial velocity distribution measured by Standing and coworkers (16) indicates a radial velocity of about 2.4 eV and a much higher axial velocity (e.g., 50 eV for bovine insulin).

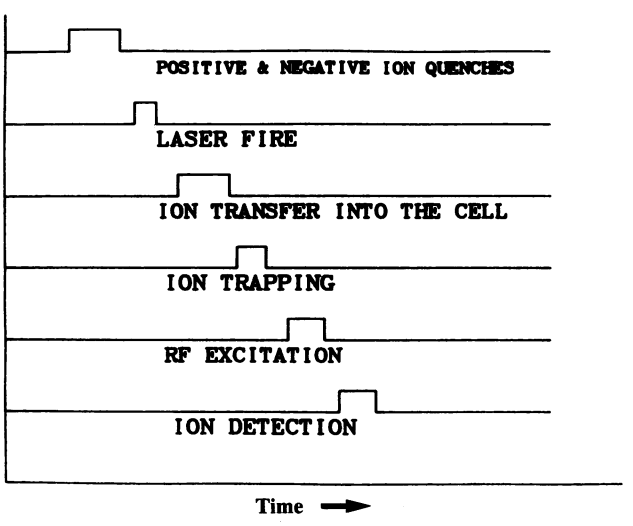

FIG. 2. Event sequence for MALD/FTICR experiments.

Fig. 4 contains the MALD/FTICR mass spectrum of gramicidin S, angiotensin I, renin substrate, and melittin. In each case the spectra were obtained at different trapping voltages, and the optimum trapping voltage increases as the mass of the analyte is increased (see Fig. 5). Note that gramicidin S is efficiently trapped at voltages >0.5 V, whereas melittin requires a minimum trapping voltage of ≈7 V. Ion yields (Fig. 5) represent relative values of ions of interest trapped in the cell and are normalized to the largest peak in the spectrum. In an effort to check the reproducibility of ion yields, at least three experiments (each experiment being an average of 50 laser shots) were performed for each data point; variations between measurements were <10%. It is interesting to note that the optimum VTR of each sample correlates reasonably well with the kinetic energy measurements reported by Beavis and Chait (14). However, the optimum VTRs change as the laser power density changes. At lower laser power densities, the number of desorbed species decreases. A decrease in the number of desorbed species results in (*i*) decreased ion yield and (*ii*) decreased number of gas phase collisions. Both conditions (*i* and *ii*) would require higher VTRs. First, higher VTRs are required to trap a measurable number of ions at decreased ion yields. Second, a decreased number of postdesorption collisions alters the translational energy distributions of desorbed particles (17–21), and higher VTRs are required.

The effects of using high VTRs to trap high kinetic energy ions are manifested in two ways. First, the absolute abundance of high m/z $[M + H]^+$ ions changes with VTRs. It is worth noting that the absolute signal intensity increases as the VTR is increased beyond the minimum voltages required for trapping a particular ion (i.e., larger numbers of ions are trapped in the ICR cell as the VTRs are increased). For instance, trapping of melittin $[M + H]^+$ ions requires +7 V, and increasing the VTR results in an increase in the $[M + H]^+$ ion signal (Fig. 5). On the other hand, at much higher VTR (e.g., greater than +20 V), ion signals are lost. The higher voltage ion trapping method works well only for ions up to approximately m/z 3000. The upper mass limit for this method of ion trapping is determined by the axial ion ejection and increased magnetron radius of the trapped ions (22–24). Second, the experimentally measured ICR frequencies of ions vary with the VTRs (22, 25, 26). It has been shown that "screened" (26) and "field-corrected" (8, 27) ion traps can be used to minimize the frequency shifts induced by trapping potentials. To account

†Pan, Y. & Cotter, R. J., Measurement of Initial Translational Energies of Peptide Ions in Laser Desorption/Ionization Mass Spectrometry, *Proceedings of the 39th ASMS Conference on Mass Spectroscopy and Allied Topics*, May 19–24, 1991, Nashville, TN, pp. 364–365.

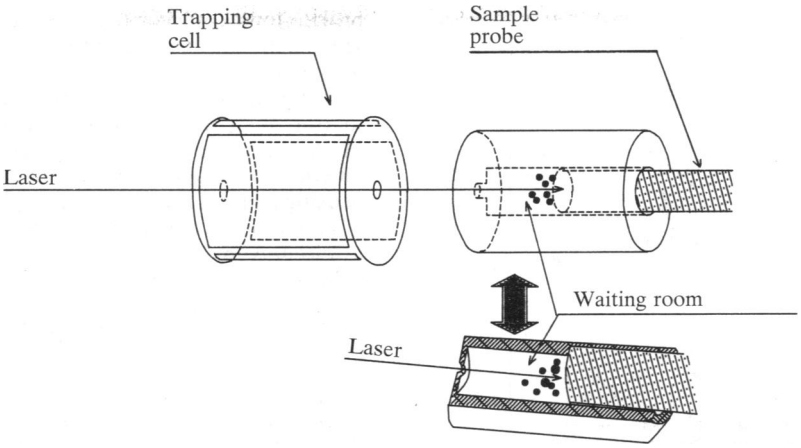

FIG. 3. (*Upper*) Diagram of the trapping cell and external waiting room with the sample probe inserted. (*Lower*) Cross section of the waiting room.

for the deviation of ICR frequency with trapping potentials, it is necessary to calibrate the mass axis with each VTR setting.

The influence of the VTR on the detectable yield for $[M + H]^+$ ions of large peptides is consistent with our previous studies that indicate that the kinetic energy of high mass ions formed by desorption ionization methods adversely affects the performance of the ICR (9). We have attempted collisional relaxation experiments by introducing a collision gas (He) through a pulsed valve to cool the translationally excited ions; however, these experiments were largely unsuccessful.

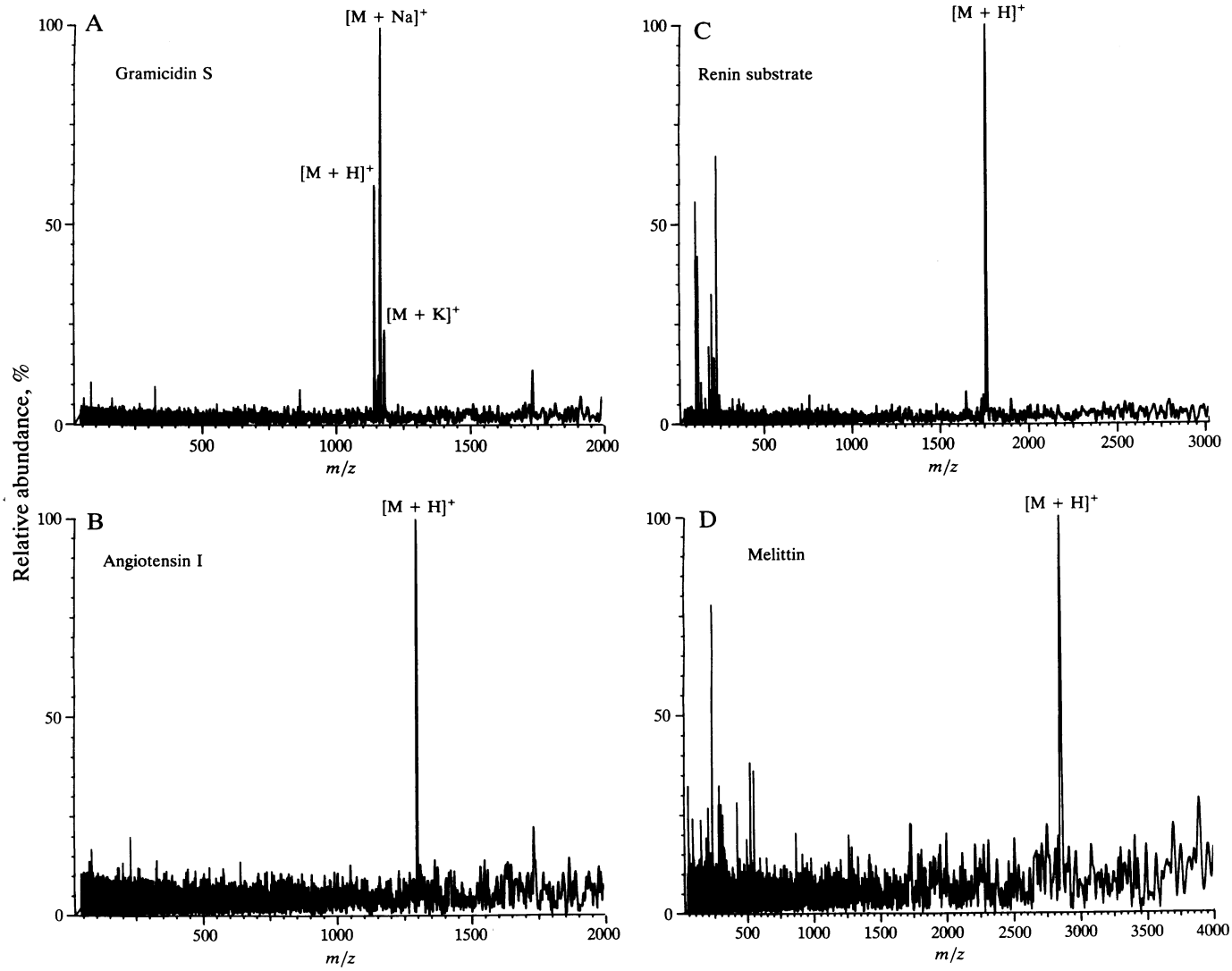

FIG. 4. MALD/FTICR mass spectra (50 scans, 32,000 data points). (*A*) Gramicidin S (m/z = 1141.5) at VTR = 1 V. (*B*) Angiotensin I (m/z = 1296.5) at VTR = 2 V. (*C*) Renin substrate (m/z = 1759.0) at VTR = 6 V. (*D*) Melittin (m/z = 2846.6) at VTR = 9 V. 4-Nitroaniline was the matrix used for each spectrum.

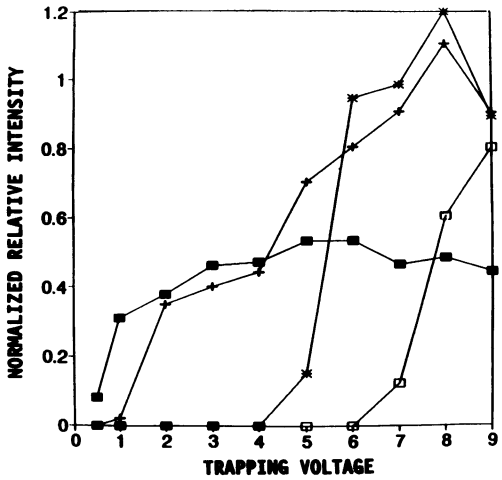

FIG. 5. The normalized relative ion abundances plotted versus VTRs for gramicidin S (■), angiotensin I (+), renin substrate (*), and melittin (□).

The failure of these pulsed-valve experiments is in part due to inefficient cooling of desorbed ions. In order to have sufficient numbers of gas phase collisions for translational cooling of desorbed ions, a large dose of reagent gas must be introduced into the ion cell. Because ion detection in FTICR requires a low vacuum (e.g., $<10^{-8}$ torr), removal of large doses of reagent gas requires longer pumping times. As the pumping time (after ion formation) increases, the probability of losing ions increases. A method that has been very successful is to perform MALD in a small chamber (waiting room). Laser ablation produces a high density of neutral and charged species, and, in a confined space ($\approx$0.25 cm^3), the pressure is sufficiently high for collisional relaxation to occur. This is the same general approach involved in the waiting room experiment of Smalley and coworkers (15).

The FTICR mass spectrum of bovine insulin shown in Fig. 6 was obtained by using the waiting room to relax translationally excited insulin ions. In this experiment, laser-desorbed species undergo collisional relaxation before entering the ion cell (Fig. 3). Collisionally thermalized ions are drifted into the cell by applying a bias voltage to the sample

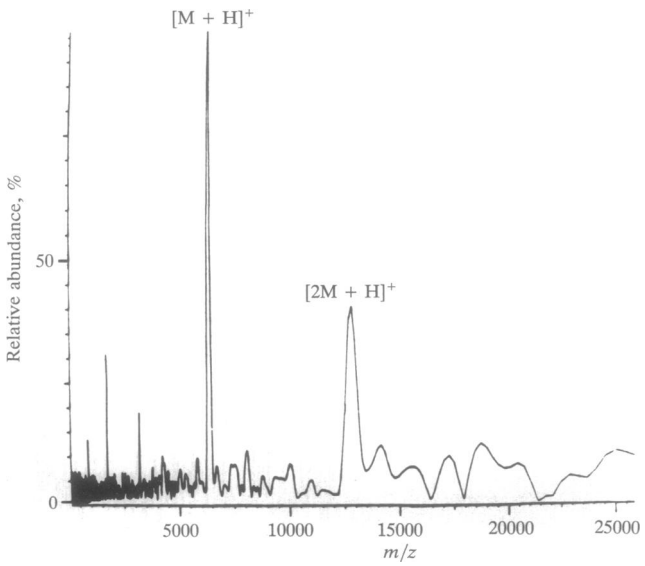

FIG. 6. FTICR mass spectrum (1 scan, 16,000 data points) of bovine insulin (m/z = 5734) obtained by using the waiting room for collisional relaxation.

probe. Ions entering the ICR cell have lost most of their kinetic energies and can be trapped at lower (less than +9 V for positive ions) voltages.

In conclusion, we have shown that high VTRs can be used to trap high mass/high kinetic energy ions produced by MALD ionization. This result is consistent with the measured kinetic energy of ions formed by MALD (14). The limitation in using high VTRs is due to complications of the ion motions (e.g., increased magnetron motion). We have also shown that to circumvent the problems associated with higher VTRs, collisional relaxation in a small volume chamber can be used effectively for trapping high mass ions. Therefore, detailed studies of high mass biomolecule ions produced by MALD ionization can be performed.

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