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Technical Notes

A Rotating Ball Inlet for On-Line MALDI Mass Spectrometry

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The rotating ball inlet (ROBIN) is presented in a new design for on-line matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). This method uses a capillary to deliver a matrix and analyte solution to the surface of a rotating ball upon which MALDI is carried out. The ball is in contact with a polymer gasket surrounding the capillary. Sample adhering to the surface of the ball is dragged past the gasket into the vacuum of the mass spectrometer where it is irradiated by a pulsed UV laser, and the resulting ions are mass-separated in a linear time-of-flight mass spectrometer. The mechanical sample introduction prevents clogging of the vacuum interface by matrix crystals or frozen solvent. Preliminary results from flow injection analysis (FIA) suggest that the new interface does not introduce a significant peak-tailing or memory effect. The system is capable of 20−30 h of continuous operation with a flow rate of 2 μL/min before cleaning of the ball is needed. With the prototype inlet, concentration detection limits are at the low micromolar level.

Mass spectrometry has revolutionized the analysis of biopolymers and other high-mass compounds during the past decade.[1] This is mainly due to the introduction of new ionization techniques such as MALDI and electrospray ionization (ESI). For many biological applications, MALDI time-of-flight (TOF) mass spectrometry has become a valuable tool because of its high sensitivity, tolerance to salts and buffers, and ability to analyze mixtures directly.[2] Unlike ESI, which is a continuous liquid introduction technique, MALDI can be used for the analysis of solid as well as liquid samples. However, due to the difficulties in transferring liquids into the low pressure of the mass spectrometer, MALDI has been used primarily for the analysis of discrete solid samples, and the technique cannot be readily combined with solution-based separation methods, such as liquid chromatography and capillary electrophoresis (CE), for on-line detection.

Several attempts have been made to develop an interface for on-line MALDI analysis of flowing liquid samples.[3] One approach makes use of continuous flow (CF) through a capillary that transports the matrix and analyte to the tip of the sample probe.[4,5] The simple design of CF probes makes it a rugged and versatile interface that can be coupled to liquid-separation devices. Only a limited number of liquid matrixes have been found suitable for CF-MALDI: 3-nitrobenzyl alcohol (3-NBA) and 2-nitrophenyl octyl ether are the only known matrixes suitable for UV CF-MALDI and ethanol absorbing at infrared-laser wavelengths has recently been demonstrated as a useful CF-MALDI matrix.[6]

Another liquid-inlet system utilizes a pneumatic nebulizer to create an aerosol for subsequent MALDI analysis.[7,8] In the aerosol MALDI method, the solution containing matrix and analyte is sprayed into the mass spectrometer where the solvent evaporates. The dried aerosol particles are ionized with a pulsed laser and analyzed by TOF MS. Aerosol MALDI is, in contrast to CF-MALDI, compatible with crystalline matrixes. The mass resolution for aerosol MALDI in TOF MS is limited by the large ion spatial distribution in the acceleration region of the ion source, but resolution has been improved by implementing an ion reflector that compensates for the spread in ion energies.[8] Another drawback is high sample consumption, which has been partly solved by introducing and ionizing single aerosol particles.[9]

Recently, a device for continuous vacuum deposition of matrix and analyte from a solution onto a moving surface inside the mass spectrometer has been reported.[10] The device makes use of a rotating quartz wheel onto which the liquid is deposited through a narrow fused silica capillary that is kept in contact with the wheel. When the wheel is rotating, deposited sample is transported into the ion source region where MALDI takes place. Promising results have been obtained, and the system is compatible with crystalline matrixes because clogging at the capillary exit is prevented due to the physical contact with the rotating wheel. A

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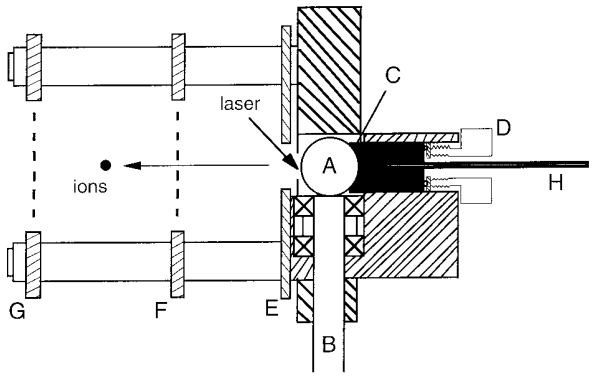

Figure 1. Diagram of the online ROBIN-MALDI probe. A, 10 mm in diameter stainless steel ball; B, drive shaft; C, gasket; D, adjustment screw; E, repeller; F, extraction grid; G, ground grid; H, capillary. The ball is rotated through the shaft, which is connected to a gear motor positioned outside the vacuum chamber (not shown).

major disadvantage of the system is the limited operation time: the wheel must be cleaned after it has made a 360-degree cycle of about 3 min.

We have developed an interface capable of continuously introducing solutions directly into a conventional MALDI-TOF MS system for a prolonged period of time. The new interface represents a development of the rotating ball inlet (ROBIN) which was originally designed for the continuous mass-spectrometric measurement of volatile species.[11−13] The principle of the ROBIN inlet is that sample adhering to the surface of a ball is continuously carried past a polymer gasket into the vacuum chamber. Volatile components evaporate from the surface of the ball when exposed to the vacuum and may be ionized by electron impact. We now show that nonvolatiles may be desorbed and ionized by laser irradiation of the ball surface in the vacuum chamber. Because the liquid sample is introduced as a thin layer there is insufficient material on one spot to form large crystals that could clog the interface. Furthermore, evaporative cooling is insufficient to cause freezing of the solvent on the ball surface, and thus, there is no risk of clogging because of ice formation.

EXPERIMENTAL SECTION

The design of the new rotating ball inlet along with the ionization region of the time-of-flight mass spectrometer is shown in Figure 1. An AISI type 420 stainless steel ball 10 mm in diameter is fitted with a shaft and attached to a gear motor. The motor (Maxon Motor, Interelectric AG, Sachseln, Switzerland) is positioned outside the vacuum chamber and connected to the shaft via a motion feed-through and nonconducting drive shaft (not shown). There are two notable differences between the present and the previously published ROBIN design for electron-impact ionization. First, the major part of the ball is inside and not outside the vacuum chamber as in the previous design. Second, the gasket is fitted with a single capillary to conduct the sample to the ball surface and not, as in the previous design, an inlet and an outlet capillary that allow flow-through sampling. These two changes are due to practical adaptions to the configuration of the mass spectrometer rather than to absolute necessities. A 75-cm-long PEEK plastic tube (1.6-mm o.d., 0.06-mm i.d) delivers the sample to the rotating ball inlet using a syringe pump (Harvard Apparatus Inc., model 55-2222, South Natick, MA). All experiments were carried out with the ball rotating at 13 rpm and a liquid flow rate of 2 μL/min. The pressure in the ion source chamber is typically at 5×10^{-5} Torr during continuous sample introduction.

Samples were made from matrix and analyte dissolved in methanol (99.8%; EMScience, Gibbstown, NJ). The matrix compound 2,5-dihydroxybenzoic acid (Sigma, St. Louis, MO) and the analytes bradykinin (B-3259; Sigma), bovine insulin (A-5500; Sigma), and bovine serum albumin (A-0281; Sigma) were used without further purification. For all the mass spectra reported, the solutions contained 10 mg/mL 2,5-dihydroxybenzoic acid (DHB) as the matrix in a methanol solvent. Various analyte concentrations were used as specified in the figure legends.

The time-of-flight mass spectrometer used in this work is an in-house-built instrument consisting of a 1-m linear flight tube equipped with delayed extraction and a dual 25-mm microchannel plate detector.[14] All experiments reported here were carried out in positive-ion mode. The instrument is typically operated with an accelerating voltage of 17 kV and an extraction pulse voltage of 3 kV. Two acceleration plates spaced 26-mm apart are positioned after the repeller. The first grid is at 17 kV, and the second grid is at ground potential. All experiments with UV laser irradiation were carried out with 2 or 10 Hz pulsed 355-nm radiation from a frequency-tripled Nd:YAG laser (Minilite, Continuum, Santa Clara, CA). Mass spectra were recorded by a 500 MHz digital oscilloscope (LeCroy 9350CM, Chestnut Ridge, NY) and transferred to a computer for further data analysis.

RESULTS AND DISCUSSION

To investigate the performance of the ROBIN MALDI interface, different solutions containing crystalline matrixes were introduced into the system under vacuum at a flow rate of 2 μL/min. It was a general observation that the system was compatible with compounds that crystallize or freeze when exposed to a vacuum, e.g., pure water and commonly used IR and UV MALDI matrixes such as succinic acid and 2,5-dihydroxybenzoic acid (DHB). Because of this finding, we decided to test the system with DHB as the matrix and a 355-nm Nd:YAG laser for MALDI.

Figure 2 shows a spectrum of bradykinin using a DHB matrix obtained using delayed extraction MALDI. The mass resolution is about 300 fwhm, which is insufficient to resolve the isotopic peaks of this peptide. A major drawback of this prototype version of ROBIN MALDI is the region around the repeller plate in the ion source. The ball is positioned 2.5 mm behind a slit in the repeller plate, giving rise to nonparallel field lines that may affect mass resolution. We are considering a future design where the ball surface is in the plane of the repeller plate, resulting in parallel field lines. The relatively high pressure (5×10^{-5} Torr) in the ion source may also affect the mass resolution due to collisions during ion acceleration. It may be possible to separately evacuate the region around the ball to give a lower pressure in the ion source, and this might also improve the mass resolution.

The spectrum of bradykinin in Figure 2 shows sodium and potassium adducts on the high-mass side of the protonated

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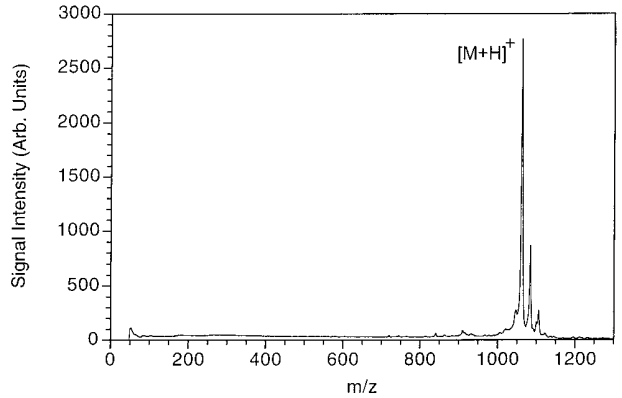

Figure 2. UV ROBIN-MALDI mass spectrum of 20 μM bradykinin. The linear TOF spectrum is a sum of 25 spectra recorded in delayed extraction mode with 17 kV acceleration voltage, 3 kV switched voltage, and a delay time of 4 μs.

molecule. Considering a mass spectrum of a larger protein, one realizes that tailing of the analyte peaks due to unresolved adduct peaks will make mass determination difficult. Organic ammonium salts have been shown to reduce adduct peaks and enhance signal intensities.[15] Figure 3 shows mass spectra covering the molecular-ion region obtained for bovine insulin with and without the addition of ammonium citrate. Although the salt adducts were still present after desalting the protein, they were reduced relative to the $[M + H]^+$ peak. Notably, the intensity of the $[M + H]^+$ peak increased in absolute intensity. An on-line desalting step may be necessary,[16] as a thin sample matrix film can give rise to more extensive alkali-adduct formation in comparison with conventional thick-layered sample preparations.[10]

In this initial work, the ROBIN MALDI system was tested for its ability to perform flow injection analysis (FIA). Figure 4 shows the flow injection ion profile of three 1-μL injections of 10 pmol insulin. The peak area of the molecular ion was integrated by the digital oscilloscope generating 10 data points per second that were transferred to the computer for further analysis. The laser was operated at a repetition rate of 10 Hz, and the ions were accelerated in static mode. No significant memory effects or peak tailing were observed, suggesting that the system can potentially be utilized in connection with separation techniques. Note, however, that the widths of the FIA peaks are broader than would be expected from the injected volume. This may be a manifestation of a limited memory effect; however, the extent of turbulent mixing on band broadening is not well-known for this system. Additionally, there is a large uncertainty in the injected volume because these preliminary studies were performed with an uncalibrated loop and an injection valve better suited to larger volumes and flow rates (model 7010, Rheodyne, Rohnert Park, CA).

Because matrix was added to both the mobile phase and the injected samples, a significant quantity of matrix was left on the ball after the flowing liquid solution was exposed to the vacuum. When the ball was examined visually after 25 h of continuous operation, small ridges of unconsumed matrix were observed bordering the track of the polymer gasket. This could explain why

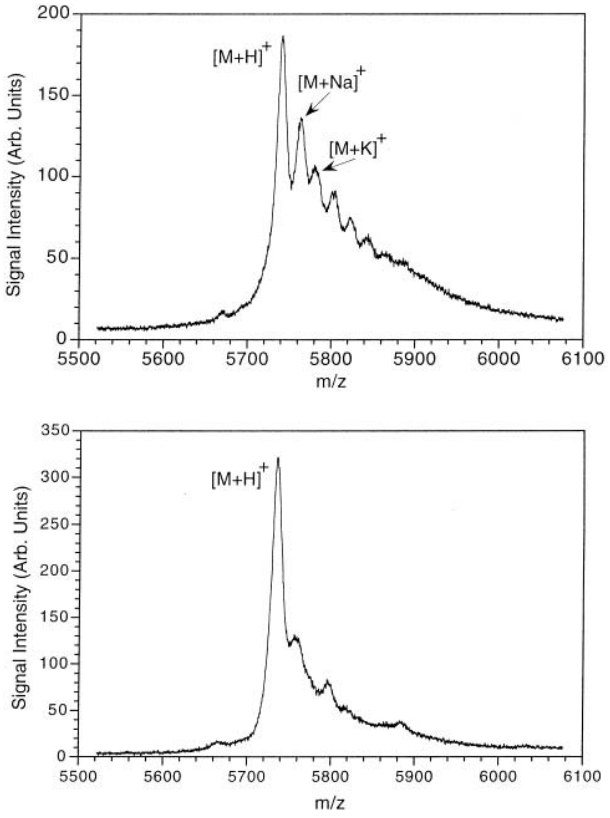

Figure 3. UV ROBIN-MALDI mass spectra of 10 μM bovine insulin (a) before and (b) after addition of ammonium citrate (1 mg/mL). Each spectrum is a sum of 25 spectra recorded in delayed-extraction mode with 17 kV acceleration voltage, 3kV switched voltage, and a delay time of 4 μs.

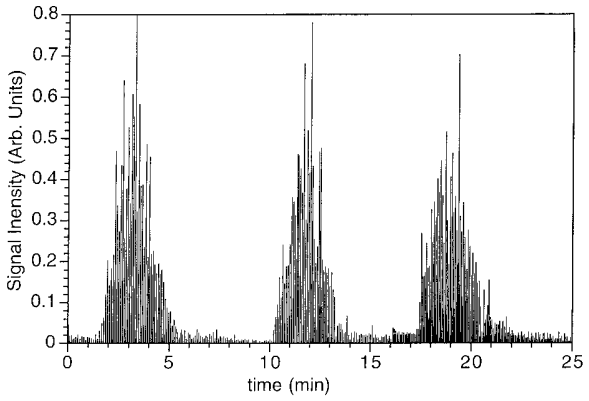

Figure 4. Flow injection ion profile of three repeated injections of 10 pmol bovine insulin. The laser was operated at a repetition rate of 10 Hz.

no extensive memory effects were seen. The microcrystalline remnants of matrix and analyte may be "snow-plowed" away from the irradiation impact zone by the gasket.

Periodic intensity variation of the ion signal, synchronous with the rotation of the ball, is observed during UV ROBIN MALDI measurements (Figure 4). These spikes may be caused by an uneven distribution of scratches or cavities on the surface of the ball, resulting in a single optimum spot on the ball circumference. We earlier obtained electron micrographs of the ball indicating that the surface is covered with evenly distributed cavities of a

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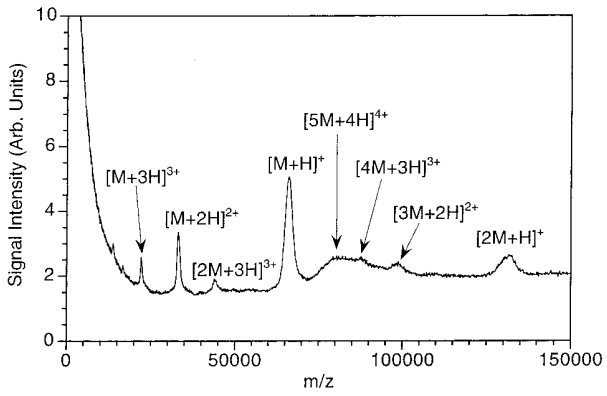

Figure 5. UV ROBIN-MALDI mass spectrum of 10 μM bovine serum albumin. The spectrum is a sum of 25 spectra recorded in static mode with 29 kV acceleration.

mean depth of 0.5 μm.[11] The cavities on the surface of the ball used in this work are probably larger because the surface has been roughened to accommodate larger sample volumes. This was done by suspending the ball in a drill press and gently touching the surface with a piece of sandpaper. We assume that these cavities are responsible for the major part of the transport of sample into the mass spectrometer and plan to manufacture a ball with homogeneously distributed cavities of a uniform depth, which may give a more regular ion signal.

The accessible mass range of the time-of-flight analyzer allowed us to detect molecules having quite large m/z values. However, for the compounds we analyzed, adduct ions could not be distinguished from the molecular ion for m/z values exceeding the mass of cytochrome *c* (M_r 12 384), where extensive overlap of multiple adduct ions reduced the mass resolution even with delayed extraction. This is in good agreement with the observations of Whittal et al. using time-lag focusing for UV CF-MALDI.[5] The ROBIN MALDI-TOF system has the ability to detect proteins of high mass, and as an example, we obtained spectra of bovine serum albumin (M_r 66 430) using static ion extraction (Figure 5). The mass resolution is remarkably reduced: about 20 fwhm compared with 300 for insulin and bradykinin. We suspect that, in addition to adducts, there is a contribution from metastable ion fragmentation, which normally hampers mass resolution in the mass range above 30 kDa.[17] Pronounced oligomerization and multiple charging is seen in the spectrum obtained from bovine serum albumin. It may be that gas-phase reactions give rise to the formation of such complexes. We suspect that the high pressure in the ionization region increases the tendency of oligomer formation. The abundances of these oligomer signals are, however, much less intense than the singly charged (monomeric) protein, making analyte identification feasible.

The spectra reported here are obtained from solutions with analyte concentrations between 10 and 20 μM. This is an upper bound to the detection limit which will be reported later in a detailed study. The sample utilization is not optimized since the contact between the ball and the polymer gasket gives a large area where sample will be deposited. We have calculated the sample utilization to be less than 5% on the basis of the ratio of the laser spot diameter and the width of the sample deposit on the ball. In future designs, the gasket (Figure 1C) and resulting sample belt around the ball will be narrowed to utilize the sample more effectively. Furthermore, we are planning to manufacture a ball with small regularly distributed grooves that may result in a more uniform sample trace.

CONCLUSIONS

The ROBIN MALDI interface provides an alternative means for the continuous introduction of liquid samples into a mass spectrometer. For biopolymers, the continuous deposition of sample may be applicable for on-line coupling of MALDI-MS with separation techniques such as liquid chromatography and capillary electrophoresis, which are typically combined with spray ionization techniques. However, MALDI offers several advantages over ESI, including a greater tolerance to impurities, spectral simplicity, and compatibility to simple and inexpensive time-of-flight mass spectrometers. Using a MALDI direct insertion probe it is often necessary to find an optimal target point on the probe tip. ROBIN MALDI overcomes this inconvenience, as the target zone is geometrically fixed.

The major limitation of the system is the mass resolution, which preferably should be 1 order of magnitude higher. Achieving a lower pressure and more parallel field lines in the ion-source region should improve the mass resolution. We also plan to implement a reflectron flight tube, which will compensate for the ion energy spread and thereby achieve further resolution improvements. Furthermore, the sample utilization should be more efficient, which can be improved by reducing the contact area between the sealing gasket and the ball.

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