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Technical Notes

A Laminar Flow Nebulizer for Aerosol MALDI

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A laminar flow nebulizer was developed for use with aerosol matrix-assisted laser desorption/ionization mass spectrometry. The nebulizer consists of a glass pneumatic nebulizer combined with a laminar flow tube. Particles formed at the pneumatic nebulizer are entrained in an auxiliary gas stream that dries the particles in the laminar flow tube. The mass range of the reflectron time-of-flight mass spectrometer has been extended to greater than 5000 Da: a mass spectrum of bovine insulin is reported at a mass resolution of 100. Mass spectra of bovine insulin and the peptides bradykinin and angiotensin II contain features resulting from prompt analyte dissociation prior to ion acceleration.

Aerosol matrix-assisted laser desorption/ionization (MALDI) was developed as a direct liquid introduction method for MALDI mass spectrometry.[1] In this method, a solution of matrix and analyte is delivered directly into the mass spectrometer as an aerosol. Ionization occurs when the aerosol particles enter the ion source and are irradiated by a pulsed ultraviolet laser. Mass separation is accomplished in either a linear[2] or reflectron[3] time-of-flight (TOF) mass spectrometer. The liquid flow rate of 0.5−1 mL/min is ideal for liquid chromatography; aerosol MALDI has been coupled to LC/MS for analysis of biomolecules[4] and synthetic polymers.[5] However, the detection limit must be improved if the method is to compete with electrospray TOF mass spectrometry used in conjunction with fast liquid separations.[6−8]

It has been suggested that ionization efficiency might be improved through more efficient desolvation of the aerosol particles.[2] Ionization of solvent-containing particles may not be efficient if laser energy is dissipated in solvent evaporation. In the current aerosol MALDI configuration, nebulization occurs in vacuum at a pressure of 0.1−1 Torr. Although the particles pass through a heated drying tube, energy transfer is probably not efficient at the reduced pressure of the nebulizer chamber. The presence of solvent cluster ions in the low-mass region of MALDI mass spectra is taken as evidence of incomplete desolvation of the particles.[2,3] Recent results have demonstrated that single aerosol particles dried at atmospheric pressure before introduction into the vacuum of the mass spectrometer show no evidence of solvent cluster peaks in the mass spectra.[9]

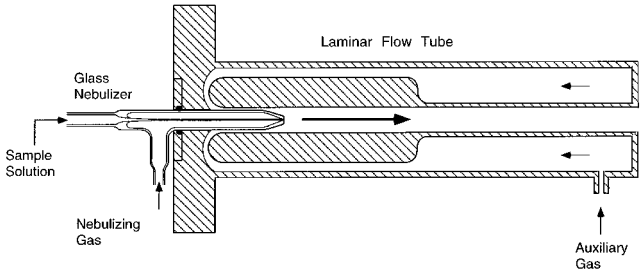

Figure 1. Schematic diagram of the laminar flow nebulizer. The aerosol is formed at the glass pneumatic nebulizer and sprayed directly into the auxiliary gas stream.

A laminar flow nebulizer for use with inductively coupled plasma mass spectrometry (ICPMS) was recently reported by French et al.[10] This nebulizer couples a droplet-on-demand (DOD) particle generator with a laminar flow tube that uses argon gas heated to 500 °C. It was found that droplets as large as 60 μm in diameter could be evaporated to dryness in a 20 cm flow tube. Advantages reported for this nebulizer configuration include decreased background signal and near complete sample utilization. The laminar flow tube configuration was chosen for an aerosol MALDI nebulizer because it allows aerosol particle drying at a higher pressure yet the nebulizer outlet remains in vacuum. Additionally, it may be possible to use the laminar flow tube to couple a DOD particle generator to aerosol MALDI. With single-aerosol MALDI, it is possible to approach full sample utilization and significantly improve detection limit.

In this paper we report results from a laminar flow pneumatic nebulizer coupled to the aerosol MALDI reflectron TOF mass spectrometer. The nebulizer was tested with the analytes bradykinin, angiotensin II, vitamin B_{12}, and bovine insulin. Spectral features revealed by the improved signal levels are discussed.

EXPERIMENTAL SECTION

The aerosol MALDI mass spectrometer used in this work was described in detail previously.[5] The sample solution of matrix and analyte is sprayed directly into the mass spectrometer using a pneumatic nebulizer. Particles are dried in a heated tube and ions are formed by a pulsed UV laser. Mass separation occurs in a two-stage reflectron TOF mass spectrometer.

A schematic diagram of the laminar flow nebulizer is show in Figure 1. The design is based on the monodisperse dried

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microparticle injector developed by French et al. for use with ICPMS.[10] The nebulizer used in this work consists of a concentric glass pneumatic nebulizer coupled to a stainless steel laminar flow tube. The glass nebulizer is a Meinhard Model SB·50·C1 (J.E. Meinhard, Santa Ana, CA). Meinhard Models SB·50·C0.5 and HEN were also used and performed similar to the Model SB·-50·C1. The glass nebulizer is attached to the flow tube with an o-ring fitting that allows a small amount of axial translation within the tube. The flow tube is constructed from a 2.75 in. conflat flange and two concentric stainless steel tubes. The outer tube is 3.5 cm o.d. and 3.2 cm i.d. and the inner tube is 7 mm i.d. The length of both tubes is approximately 23 cm. The inner tube is 2.2 cm o.d. at the nebulizer inlet with a torroidal surface of 4.5 mm radius facing the flange. A matching torroidal surface of 8 mm radius is cut into the flange itself to form a smooth channel. The nitrogen auxiliary gas enters the laminary flow tube at the tip and flows toward the nebulizer through the outer tube. The auxiliary gas flows smoothly around the nebulizer and entrains the aerosol in the flow into the central tube. The gap between the glass nebulizer and the inner flow tube is 0.5 mm. Typical operating conditions are a liquid flow rate of 0.5 mL/min and a nitrogen carrier gas flow rate of 0.6 L/min for the nebulizer and 2.5 L/min for the auxiliary flow.

The ionization laser was a frequency-tripled 10 Hz Nd:YAG operating at 355 nm with a pulse energy between 100 and 120 mJ. Laser irradiance was between 3 and 4 GW/cm^2 calculated using the focused beam size of 0.1 by 5 mm at the 50% irradiance contour.[3] The mass spectra shown in this article resulted from an average over 500 laser shots with a 2 ns data point spacing. Spectra were boxcar averaged for plotting.[3]

Sample solutions were made from matrix and analyte dissolved in methanol (99.8%, E. M. Science, Gibbstown, NJ) with 6% trifluoroacetic acid (J. T. Baker, Pittsburgh, NJ) by volume. The matrix compounds α-cyano-4-hydroxycinnamic acid (Sigma, St. Louis, MO) and 2,5-dihydroxybenzoic acid (gentisic acid, Sigma), the analytes bradykinin (84%, B-120, RBI, Natick, MA), angiotensin II (A-9525, 98%, Sigma), and vitamin B_{12} (V-2876, 99%, Sigma), and bovine insulin (I-5500, Sigma) were used without further purification. Bradykinin, angiotensin II, and vitamin B_{12} solutions contained 1 mg/mL analyte and 5 mg/mL matrix. The bovine insulin solution contained 2 mg/mL analyte and 10 mg/mL matrix.

RESULTS AND DISCUSSION

The laminar flow nebulizer was tested with the analytes bradykinin, angiotensin II, and vitamin B_{12}, which have been studied previously with the reflectron instrument.[3,11] Bovine insulin, which has been studied extensively with linear TOF aerosol MALDI,[2] was used to test mass spectrometer performance with higher mass analytes.

An aerosol MALDI mass spectrum of the peptide bradykinin with the laminar flow nebulizer is shown in Figure 2. The matrix was 2,5-dihydroxybenzoic acid (DHB). The matrix α-cyano-4-hydroxycinnamic acid gave a larger protonated molecule signal, but also extensive solvent clusters. The peak at m/z 1061 corresponds to protonated bradykinin $[M + H]^+$ at a mass resolution of $m/\Delta m = 400$ fwhm. For spectra obtained with the same matrix and pulse energy, the area of the protonated bradykinin peak is 3−5 times larger with the laminar flow tube than with the pneumatic nebulizer. The improved performance of the laminar flow nebulizer appears to be general: an approximate factor of 3−5 increase in signal intensity was observed for the analytes angiotensin II and vitamin B_{12}. Other spectral features are similar to those observed previously on this apparatus with a simple pneumatic nebulizer.[3] The peak immediately to the left of the $[M + H]^+$ peak corresponds to postsource decay (PSD) loss of NH_3 from bradykinin, forming the z_9 fragment. The peak to the right of the $[M + H]^+$ peak at m/z 1175 corresponds to an adduct of trifluoroacetic acid with bradykinin which has been observed previously.[3] Peaks in the low-mass region up to m/z 500 result primarily from protonated methanol clusters, $H^+(CH_3OH)_n$, and protonated methanol clusters containing one or more water molecules.

Peaks in the region from m/z 500 to 1050 in Figure 2 result from both prompt and postsource decay fragments (excepting the $[M + 2H]^{2+}$ peak at m/z 531). Figure 3 shows an expanded view of the intermediate m/z region of the spectrum in Figure 2. The PSD peaks can be easily identified because they shift in apparent

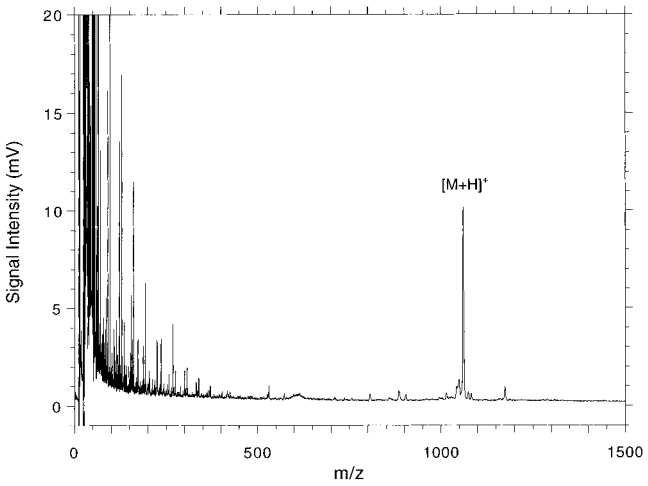

Figure 2. Aerosol MALDI reflectron TOF mass spectra of bradykinin with DHB matrix obtained with the laminar flow nebulizer. The laser irradiance was 3 GW/cm^2.

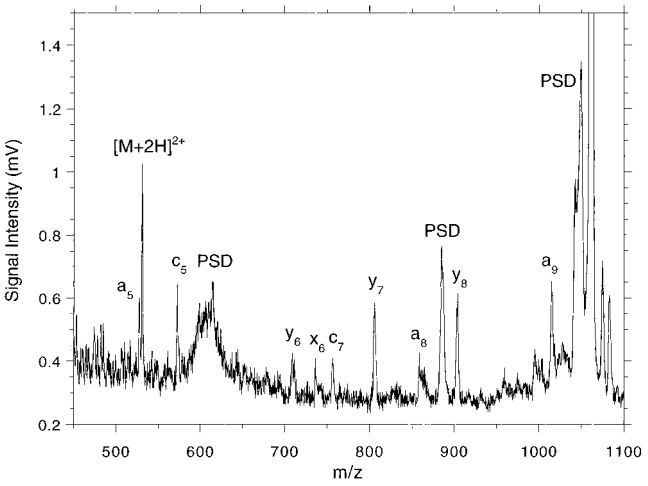

Figure 3. Aerosol MALDI mass spectrum of bradykinin showing fragment peaks. Prompt fragment peaks are labeled using the standard nomenclature. Peaks associated with postsource decay peaks are labeled PSD.

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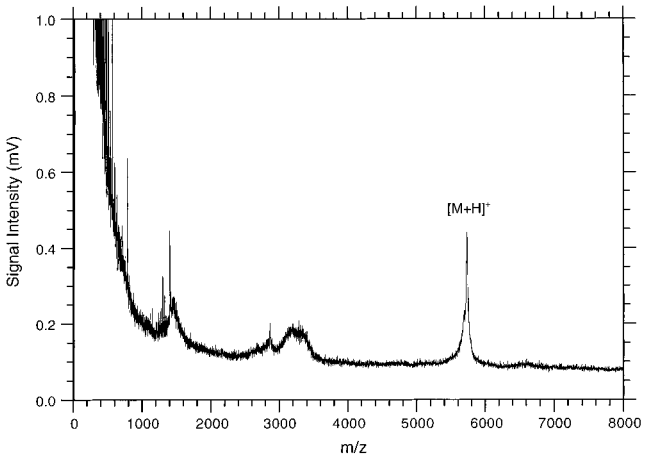

Figure 4. Aerosol MALDI reflectron mass spectrum of bovine insulin with a α-cyano-4-hydroxycinnamic acid matrix. The laser irradiance was 4 GW/cm^2.

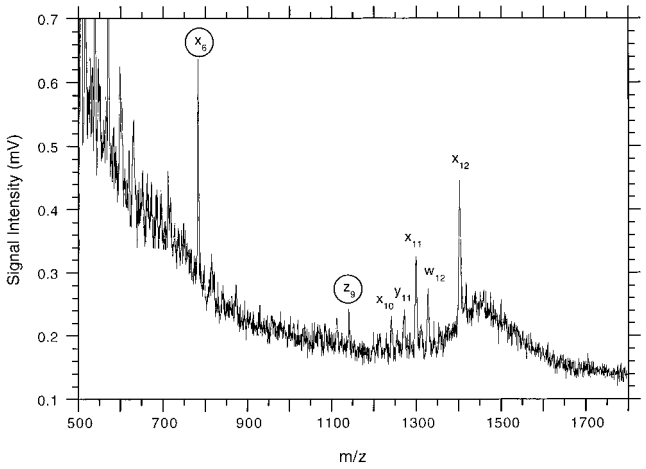

Figure 5. Aerosol MALDI mass spectrum of bovine insulin showing prompt fragmentation peaks. The circled labels indicate chain A fragments and the remaining labeled peaks correspond to chain B fragments.

m/z when the reflectron voltage is changed.[3,11,12] Three peaks in Figure 3 are associated with PSD: the PSD z_9 peak at 1050, the broad "junk hill" near m/z 600 that results from low-mass PSD fragment ions, and a resolved PSD peak at m/z 885. A simple calculation suggests that the latter peak results from y_7 ion which is formed by PSD fragmentation of the Pro−Pro bond. The y_8 fragment is not observed, although the y_8 fragment is prominent in CID mass spectra of bradykinin generated by fast atom bombardment.[13] A single intense y_7 fragment has been observed in the CID spectrum of protonated bradykinin formed by field desorption.[14]

The majority of the peaks in the region from m/z 500 to 1050 result from ion source fragmentation. These peaks are assigned as prompt fragments rather than PSD because (1) they appear at m/z values within experimental error of bradykinin fragments observed in fast atom bombardment mass spectra,[13−16] (2) the m/z value remains constant at different reflectron voltage settings, (3) some of the peaks occur at shorter flight times than the PSD junk hill. Thermal degradation in the drying tube is ruled out because mass spectra obtained with the drying tube at room temperature still give fragment peaks. The assignment and m/z values for the labeled peak are a_5 (527.5) c_5 (572.5), y_6 (710.7), x_6 (736.7), c_7 (756.6), y_7 (806.2), a_8 (858.9), y_8 (903.6), and a_9 (1015.0). The estimated mass measurement accuracy is 0.1%. Prompt fragment ion peaks were also observed in aerosol MALDI mass spectra of angiotensin II.

An aerosol MALDI reflectron TOF mass spectrum of bovine insulin (M_r = 5733.5) obtained using the laminar flow nebulizer is shown in Figure 4. Although signal from bovine insulin and larger analytes has been observed with a linear TOF aerosol MALDI mass spectrometer,[2] analytes larger than a few thousand daltons in mass have until now not been observed in the reflectron TOF instrument.[3,5] The protonated bovine insulin molecule [M + H]$^+$ is the most intense high-mass peak in the mass spectrum. The fwhm mass resolution is 100, although it appears that a shoulder peak on the low-mass side of the [M + H]$^+$ peak may be contributing to the peak width. The observed mass resolution is more than 1 order of magnitude better than that achieved for aerosol MALDI bovine insulin on a linear TOF.[2] The 10-fold improvement is similar to that observed for smaller analytes.[3] Other resolved peaks in the spectrum are the [M + 2H]$^{2+}$ peak at m/z 2868 and prompt fragment peaks between m/z 700 and 1400. The broad features at m/z 1400 and 3200 are likely associated with PSD of insulin and doubly charged insulin.

Figure 5 is an expanded region of the bovine insulin spectrum in the mass range from m/z 500 to 1800. Several fragments resulting from backbone cleavage of the bovine insulin are observed in this region. These peaks are due to prompt fragmentation: the measured masses are within experimental error of predicted backbone cleavage fragment masses, the peaks do not shift in m/z when the reflectron voltage is changed, and all peaks appear at lower flight times than any of the unresolved PSD peaks. The largest fragments and their measured masses are x_6 (783.3) and z_9 (1142.3) from insulin A chain and x_{10} (1242.6), y_{11} (1272.6), x_{11} (1300.0), w_{12} (1328.8), and x_{12} (1403.0) from B chain. The B chain fragments are a result of bond cleavage in the vicinity of the disulfide bridge closest to the B chain c-terminus. Like the bradykinin prompt fragmentation peaks, the insulin fragment peaks are observed when the drying tube is at room temperature, tending to rule out thermal decomposition prior to ionization.

Ion source fragmentation is typically not observed in MALDI TOF mass spectrometry unless delayed extraction is employed.[17] In aerosol MALDI, much higher laser energies can be used and therefore additional fragmentation mechanisms may come into play. It may be possible that photofragmentation is taking place and that residual solvent evaporation removes excess energy before complete fragmentation can occur. A recent aerosol MALDI study of vitamin B_{12} fragmentation suggests that analyte collisional activation may be an important mechanism for analyte fragmentation.[11]

CONCLUSIONS

The laminar flow nebulizer is a more efficient nebulizer for aerosol MALDI resulting in improved ion signal and increased

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mass range. Ion signal for the analytes bradykinin, angiotensin II, and vitamin B_{12} is 3−5 times larger with the laminar flow nebulizer than with a simple pneumatic nebulizer. The protein bovine insulin, which cannot be detected in the reflectron TOF apparatus when a simple pneumatic nebulizer is used, was observed at a mass resolution of 100 in the reflectron TOF apparatus. The mass resolution for bovine insulin is more than a factor of 10 greater than the resolution obtained in a linear TOF.[2] Sequence-specific fragments of bradykinin, angiotensin II, and insulin are observed in the aerosol MALDI mass spectra. The majority of the peaks observed arise from fragmentation prior to ion acceleration. Future work will be directed toward coupling a single droplet aerosol generator with the laminar flow tube.

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