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SCIENCE DIRECT

Microchemical Journal 81 (2005) 108–116

MICROCHEMICAL JOURNAL

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Mobility spectrometry of amino acids and peptides with matrix assisted laser desorption and ionization in air at ambient pressure

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Received 22 January 2004; accepted 21 January 2005
Available online 29 March 2005

Abstract

Gas phase ions for valine, glutamate, phenylalanine, angiotensin, bradykinin, LH-RH, and bombesin were formed through matrix assisted laser desorption-ionization (MALDI) in air at ambient pressure and were characterized by ion mobility spectrometry (IMS). The IMS drift tube was operated at 100 °C with air as the drift gas and without an ion shutter. Responses were obtained using α-cyano-4-hydroxycinnamic acid as the matrix and a Nd-YAG laser at 355 nm with an unfocused beam at 6 mJ per pulse and 7 mm^2 cross section. Matrix and analyte were applied to a borosilicate glass target and microgram amounts of sample provided responses lasting 10 to 15 s with the laser operated at 11 Hz. Detection limits for the peptides were estimated to be 10 to 100 pmol per laser shot. The mobility spectra for individual amino acids and peptides exhibited multiple peaks with spectral distortions and raised baselines. These features and calculated values for reduced mobilities were consistent with the existence of clusters between analyte ions and matrix neutrals and the dissociation of these clusters in the drift region of the analyzer. Mobility spectra with distinctive peaks were not obtained for MALDI-IMS of peptides larger than 5700 amu, though ion formation was suggested from the depletion of matrix signal.

Keywords: MALDI; Ambient pressure; Mobility spectrometry; Peptides; Amino acids

1. Introduction

The use of instrumental methods, particularly mass spectrometry, for biomolecule measurements has grown during the past fifteen years with interests in proteomics and the development of specialized ion sources including electrospray ionization (ESI) and matrix assisted laser desorption-ionization (MALDI) [1,2]. Through these ionization techniques, mass spectrometry has emerged as a bioanalytical tool with a broad applications for the determination of protein levels [3], the separation of mixtures of protein digests [2], and the characterization of micro-organisms [4,5]. Molecular masses up to 1,000,000 and attomole detection limits have been achieved with these ionization methods where molecules are converted without decomposition to gas phase ions [2]. In MALDI, a sample is mixed with a photoactive substance, or matrix, and deposited on a solid surface. The matrix is selected for photo-absorptivity and the tendency to undergo desorption and ionization from the solid surface when exposed to a pulsed laser (typically at 337 or 355 nm). When the mixture is irradiated, a plume of material is desorbed from the surface including neutral sample and ions from the matrix chemical. Ionization of the sample through proton transfers leads to the production of gas phase analyte ions.

Matrix assisted laser desorption and ionization is largely non-destructive toward biological molecules since the matrix absorbs much of the laser energy. Consequently, involatile labile biological analytes can often be transferred to the gas phase as intact molecular ions. Ions formed in this way are predominately singly charged or simple clusters of ions and matrix molecules, providing simple responses in comparison to ESI which multiple charge states may exist for each analyte. Analytical complications may occur in MALDI through signal suppression arising from changes in

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0026-265X/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.microc.2005.01.005

sample preparations [6], to low ion yields, and transient nature of signals.

Though MALDI has been used under vacuum with mass spectrometers, the preparation of sample and ionization at ambient pressure would facilitate continuous atmospheric monitoring and convenience of analysis. Indeed, MALDI at ambient (also called atmospheric) pressure (AP) has been demonstrated for peptides with molar masses up to 6000 amu by TOF-MS [7] or ion trap-MS [8]. Reduced fragmentation was observed with AP-MALDI compared to vacuum-based MALDI; presumably, collisions at elevated pressure assisted in stabilizing excited ions. Unfortunately, detection limits with AP-MALDI were poor in comparison to MALDI in vacuum and this was attributed to losses during ion sampling at the pin-hole interface between ambient pressure and high vacuum of the mass spectrometer. An expected, increase in the formation of cluster ions between sample ions and matrix neutrals was observed and the level of cluster formation appeared to increase with analyte mass, leading to a decrease in sensitivity for large analytes, apparently by dispersal of charge among a number of smaller peaks [7]. Nonetheless, findings from AP-MALDI-MS experiments demonstrated that matrix materials and sample preparation methods were transferable from vacuum-based procedures to ambient pressures.

The ion losses in the interface with a mass spectrometer may be eliminated or substantially reduced should ion characterization also occur at ambient or elevated pressures. Mobility spectrometers can be used to characterize ions in drift tubes operated at elevated pressures and can permit ion evaluation on the basis of size-to-charge rather than mass-to-charge ratio [9]. Such measurements have demonstrated value with biological molecules through electrospray ionization [10–13], though some of these measurements have been at reduced pressures, e.g. <10 Torr in helium atmospheres [12,13]. Response was obtained for analytes with molar masses up to 14,000 amu where ions clusters between matrix and analyte and proton-bound dimers of analyte were observed [14]. Three reports of MALDI at ambient pressure with IMS have proven that ions can be made at ambient pressure and characterized in a mobility spectrometer. Studies included an examination of the role of variables on drift times [15], the adaption of solid phase microextraction probes for MALDI-IMS [16], and the addition of a supplemental source of ions to boost signal in an MALDI-IMS-MS experiment [17]. These reports demonstrate that there may be analytical benefit from such instruments though such expectation however might be complicated by ion clustering events that could be pronounced and debilitating at ambient pressure.

In the present study, ambient pressure MALDI was combined with an IMS drift tube and explored for spectral quality, quantitative response and limits of mass for detectable ions with air as a supporting atmosphere. The use of a drift tube at elevated temperatures was intended to reduce deleterious effects from clustering reactions. Studies were begun using amino acids (< 200 amu) and extended to small peptides (~1000 amu) and large peptides (> 5700 amu).

2. Experimental

2.1. Instrumentation

The IMS drift tube is a conventional linear design with alternating rings of conductors and Teflon insulators as described previously [19] and includes openings in the source region to allow the introduction of a sample and the entrance and exit of a laser beam as shown in Fig. 1. In this design, the laser beam strikes the flat end of the sample probe at 30° and reflected radiation exits through the second window. The beam entrance and exit ports were sealed with fused silica windows (Melles Griot, CA) and a Swagelok fitting was used to hold and pneumatically seal the sample probe in the source region. Drift gas was zero air passed through molecular sieve to a moisture level of ~5 ppm. This gas was introduced at the detector end of the drift tube and passed through the tube at 130 cm^3 min^{-1} at a gas temperature of 100 °C. Ambient pressure was 665 Torr. Electric fields in the various regions of the drift tube (see Fig. 1) were the following: repeller (pre-source) region, 1.14 cm long, 509 V cm^{-1}; source region, 2.16 cm, 639 V cm^{-1}; drift region (ion shutter-to-aperture grid), 5.25 cm, 456 V cm^{-1}; and aperture grid-to-detector, 0.25 cm, 286 V cm^{-1}. Average electric field in the drift tube was 495 V cm^{-1}, with sample probe-detector distance of 7.0 cm. The ion shutters were biased open and all studies were performed with detection of positive ions.

Signal averaging and storage were performed using a PC equipped with an interface card (PCI-6024E, National Instruments, Austin, TX) and a software system developed in-house using the LabVIEW package (v.5.1, National Instruments). Each time the laser fired, an electronic pulse was produced beginning spectral acquisition. Data acquisition parameters were 1000 data points collected per spectrum at frequencies between 31 and 13 KHz (depending on the maximum drift time required), with 16 spectra averaged for each file saved (i.e., 16 laser shots).

The laser was an Nd-YAG (MiniLite II, Continuum, Santa Clara, CA) with frequency tripled output (λ=355 nm). The laser was operated at 11 Hz with pulse width <5 ns, and the beam energy was 6 mJ (calibrated with a pyroelectric head joulemeter, Molectron, Portland, OR). The beam diameter was estimated at ca. 2.7 mm using thermal paper, which suggested that the beam was a comparable size to the sample target, presented to the beam as a rod with ca. 3 mm diameter. This means that the whole target would be exposed to the laser simultaneously, in contrast to standard MALDI methodology where a focused beam is tracked across the target in search of favorable response.