Generation of Multiply Charged Peptides and Proteins by Radio Frequency Acoustic Desorption and Ionization for Mass Spectrometric Detection

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The design and implementation of a radio frequency acoustic desorption ionization (RADIO) source has been demonstrated for the analysis of multiply charged peptides and proteins. One μL aliquots of melittin, BNP-32, and ubiquitin ($\sim$1 μg of analyte) were deposited onto a quartz crystal microbalance (QCM) electrode before radio frequency actuation for desorption. Continuous electrospray parallel to/above the sampling surface enabled the ionization of desorbed species. Detection by a hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometer confirmed the intact and dissociated species observed during MS and MS/MS experiments, respectively. (J Am Soc Mass Spectrom 2009, 20, 597–600) © 2009 American Society for Mass Spectrometry

Numerous ionization techniques are being developed to address a diverse collection of complex and interesting questions by mass spectrometry (MS). Several recently developed ionization sources indirectly introduce principles of the RADIO source. Laser-induced acoustic desorption (LIAD) [1–4] demonstrates the soft desorption of analyte using a shock wave generated by a laser pulse and the potential to couple post-desorption ionization techniques such as chemical ionization at atmospheric pressure. Array of micro-machined ultrasonic electrosprays [5–7] (AMUSE), applies an rf signal to a ceramic piezoelectric transducer with a solution cavity between a micromachined silicone nozzle array for droplet-on-demand generation. The preceding techniques address RADIO's desorption mechanism. Several other techniques are mentioned below as they utilize postdesorption ionization (as in RADIO). Secondary electrospray ionization (SESI) [8–11] involves the gas-phase interaction of charged ESI droplets with neutral sample molecules for analysis by ion mobility spectrometry (IMS) or MS. Fused droplet electrospray ionization (FD-ESI) [12, 13] aerosolizes the sample solution via a nebulizer for interaction with a highly charged acidic methanol solution and reduces interferences from buffers and complex mixtures. Extractive electrospray ionization (EESI) [14–18] employs two nebulizing sprayers, one with ESI solvents and a second containing the analyte of interest for a liquid–liquid extraction process to reduce interferences from complex mixtures.

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Closely related to RADIO due to step-wise desorption with subsequent ionization are electrospray-assisted laser desorption ionization (ELDI) [19] and solid-state matrix assisted laser desorption electrospray ionization (ss-MALDESI) [20, 21]. ss-MALDESI utilizes a UV or IR laser for analyte desorption and ESI for post-ionization.

The desorption phenomena can be attributed in part to the shock wave desorption observed in LIAD, however for RADIO, the transfer of energy is accomplished by applying an rf waveform to a piezoelectric material (analogous to AMUSE). Postdesorption electrospray is the proposed ionization pathway as in SESI, FD-ESI, EESI, and ss-MALDESI.

Experimental

Materials

Melittin, BNP-32, ubiquitin, and formic acid were obtained from Sigma Aldrich (St. Louis, MO). HPLC grade acetonitrile and water were purchased from Burdick and Jackson (Muskegon, MI). Nitrogen (99.98%) and LTQ helium bath gas (99.999%) were obtained from MWSC High Purity Gases (Raleigh, NC).

Methods

Electrospray solutions consisted of acetonitrile:water (50:50% by volume) with 0.1% formic acid. Melittin was diluted to 347 μM in water, BNP-32 was diluted to 300 μM in water with 0.1% formic acid, and ubiquitin was diluted to 99 μM in 50:50:0.1% water:acetonitrile:formic acid. QCM substrates were spotted with 1 μL of the

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respective analyte immediately before analysis. This resulted in ~1 μg of analyte on the surface with a minute fraction sampled by the mass spectrometer.

Mass Spectrometer

Mass spectra were acquired on a hybrid LTQ-FT-ICR mass spectrometer (Thermo Fisher, San Jose, CA) equipped with an Oxford Instruments actively shielded 7T superconducting magnet (Concord, MA) operating in positive ion, FT mode. The injection time was set to 750 ms (BNP-32 and ubiquitin) and 500 ms (melittin) for full scan experiments (AGC off). For MS/MS, the injection time was set to 1000 ms (AGC off); product ions generated in the ion trap were transferred to the FT-ICR cell for detection. The capillary temperature was 250 °C. The standard MS capillary was replaced with a 0.5 mm i.d. × 131 mm stainless steel capillary that protruded 32 mm from the ferrule.

RADIO Source

The experiments utilized an AG 1020 "Linear" rf Amplifier/Generator (P/N AG1020; T and C Power Conversion, Inc., Rochester, NY) to actuate a quartz crystal microbalance (QCM) electrode (P/N 151689-10; International Crystal Manufacturing, Inc., Oklahoma City, OK). The surface of the QCM electrodes were Au plated Cr. Figure 1 displays the RADIO source components. The rf amplifier is shown connected to the QCM electrode with the electrospray emitter positioned above the sample spot such that the ESI plume is directed into the mass spectrometer. QCM electrodes were mounted on a Teflon bracket attached to an XYZ stage for position adjustment. The rf amplifier was connected to the QCM electrode via a BNC cable with mini-clip jumper wires. The resonance frequency to generate

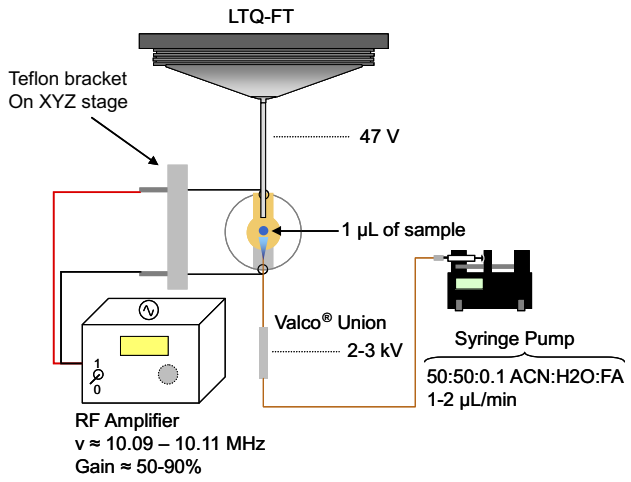

Figure 1. Schematic of the RADIO source showing the experimental configuration with the rf amplifier attached to the QCM electrode; the ESI emitter is located above the QCM electrode with flow directed at the MS inlet.

progeny droplets from a 1 μL sample on the QCM surface was experimentally modulated. At an applied rf of 10.09 to 10.11 MHz, and a gain of 60% to 90%, mass spectrometric signal was observed. No DC bias was applied to the QCM.

Solvent was electrosprayed continuously above the electrode surface during MS analysis for droplet interaction and ionization with the electrospray plume (vide infra). The electrospray parameters were 1–2 μL/min through a 30 μm electrospray emitter biased at 2 to 3 kV. The mass spectrometer inlet was biased at 47 V. The QCM electrode was cleaned between analyte depositions and subsequent acquisitions by rinsing 10 μL of 50:50:0.1% water:acetonitrile:formic acid and drying the surface with compressed air.

Control experiments were performed to ensure that signal resulted from droplet generation off of the QCM surface. The first involved turning off the rf amplifier with a sample droplet present on the surface, the second involved entirely removing the QCM electrode, and in a third, a 1 μL aliquot of solvent was deposited onto the QCM surface and the rf amplifier signal was turned on. During each control experiment, a continuous stream of solvent was electrosprayed and the MS signal was monitored with no detection of analyte. Preventative cleaning of the ESI emitter and MS inlet with an acetonitrile soaked cotton swab prevented cross-contamination between samples.

Results and Discussion

The RADIO source is illustrated in Figure 1. The positioning of the QCM electrode with respect to the MS inlet and ESI emitter was facilitated by a XYZ stage. Constant spray current was observed with intense spikes of signal on a total ion chronogram when desorbed species were detected by the mass spectrometer (data not shown).

Full scan mass spectra were obtained by depositing 1 μL of analyte before rf actuation of the QCM electrode. By constantly electrospraying parallel to the sample surface (above the analyte spot) during the desorption event, desorbed species interacted with the ESI droplets for ionization. This is analogous to ionization in fused droplet-electrospray ionization (FD-ESI) [12, 13] and extractive electrospray ionization (EESI) [14–18] due to neutral interaction with an ESI plume. Previous reports demonstrated that post-desorption ionization offers the advantage of withstanding high salt tolerances [12]. The interaction time of desorbed species with the ESI plume is one factor that can be attenuated by adjusting the linear distance of the ESI emitter from the MS inlet (holding the sample spot constant). For these experiments, an ESI emitter to MS inlet distance of ~5 mm was utilized.

Figure 2 demonstrates multiple-charging, high signal-to-noise, and isotopic resolution of brain natriuretic peptide-32 (BNP-32) [22] attained using RADIO. The

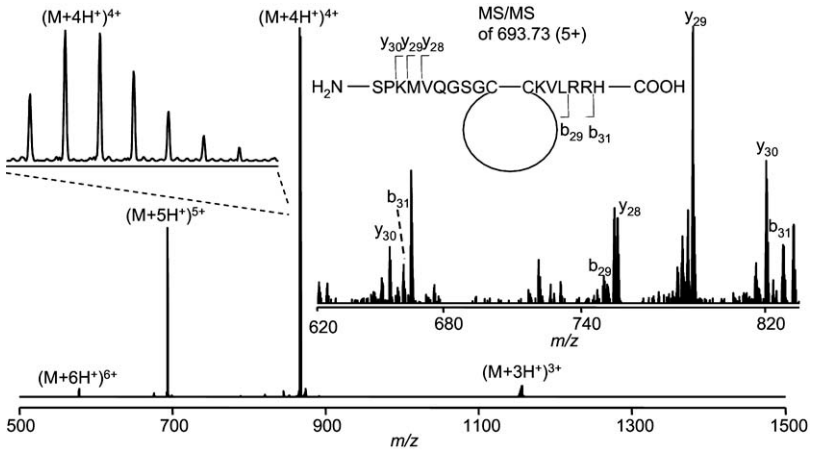

Figure 2. Intact FT-ICR detection of BNP-32 with isotopic distribution of the 4+ charge state and inset of FT-ICR detection of CID fragmentation of 5+ charge state.

3+ through 6+ charge states are shown with a zoom-in of the isotopic distribution of the 4+ charge state. Accurate mass measurements indicated that BNP-32 was oxidized as reported previously by Hawkridge et al. [22] This is a result of a cysteine–cysteine disulfide bond linkage, which forms a ring-like structure (see sequence depiction in Figure 2). MS/MS was carried out on the 4+ charge state. A representative collision induced dissociation (CID) spectra is inset in Figure 2. The number of product ions observed is attributed in part to the oxidized form of BNP-32.

Full scan mass spectra were obtained from a 1 μL sample of ubiquitin deposited onto the QCM electrode. A representative mass spectrum is shown in Figure 3 including the charge states of 6+ through 12+. MS/MS data of the 7+ precursor ion is inset in Figure 3. Additional data were collected including full scan detection of the 3+ and 4+ charge states of melittin (data not shown).

Conclusions

An ambient ionization source with multiple-charging for MS and MS/MS analyses has been described. The use of a rf amplifier for analyte desorption should enable a variety of ambient ionization mass spectrometry applications. This proof-of-principle demonstration of the RADIO source should pique the interest of investigators as a method to address complex biological questions.

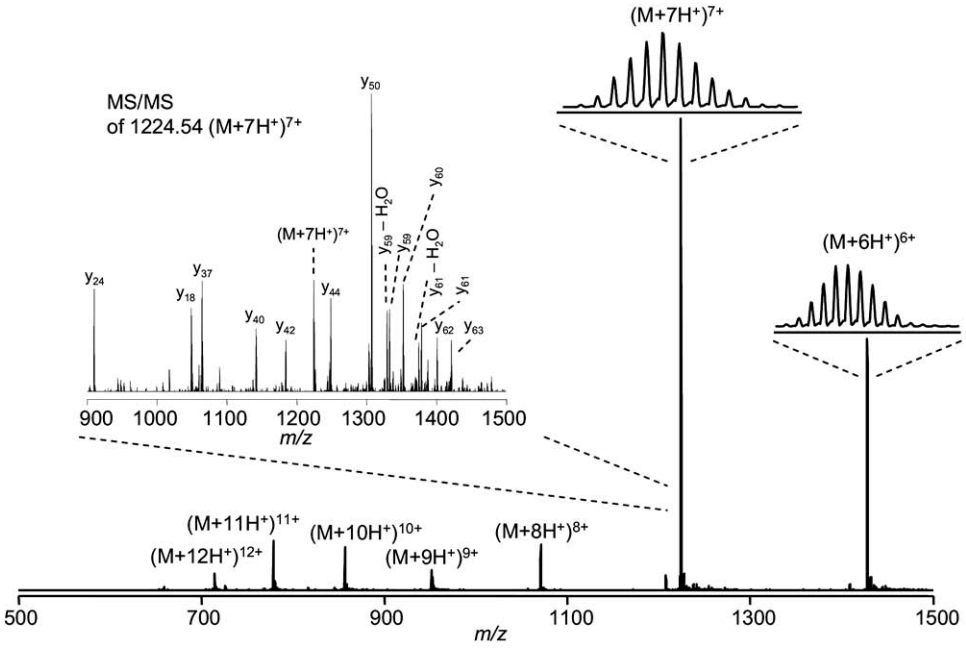

Figure 3. Intact FT-ICR detection of ubiquitin with isotopic distribution of the 6+ and 7+ charge states and inset of FT-ICR detection of CID fragmentation of 7+ charge state.

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