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Roadmap for fragmentation of protonated methylarginines in the gas phase

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ABSTRACT

Up to MS^4 collision-induced fragmentation study of protonated pseudomolecular ions of L-arginine (Arg), ω-N^G-monomethylarginine (MMA), ω-N^G,N^G-asymmetric dimethylarginine (aDMA) and ω-N^G,$N^{G'}$-symmetric dimethylarginine (sDMA) were performed with Fourier transform ion cyclotron resonance mass spectrometry. The parent pseudomolecular ions were generated in a home-built atmospheric-pressure matrix-assisted laser desorption/ionization source, and the admixing of nitrocellulose with the matrix significantly reduced the interfering matrix peaks in the resulting mass spectra. While protonated isobaric molecules (aDMA, sDMA) could be easily distinguished by low-order tandem mass spectrometry, MS^4 was needed for unequivocal revelation of some previously unreported fragmentation pathways. Among a total of 44 fragment ions generated from the two isomeric DMAs, 5 were uniquely and exclusively produced from just one of these precursor ions that allowed the precursor ion structures to be differentiated. The unsurpassed advantage of high resolution and mass accuracy of FTICR MS enabled us to identify fragmentation channels that yield fragment ions differing in mass by only ~0.01 Da. The sub-ppm mass accuracies facilitated in proposing chemical structures for all the fragments observed. Many hitherto unreported isobaric fragments from the methylated arginines are observed for the first time.
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1. Introduction

The activity of proteins are controlled or modulated by post-translational modifications. Protein methylation is an important metabolic pathway that modifies the amino acid L-arginine [1]. While methylation of arginine had been discovered more than three decades ago, its biological functions in protein sorting, transcription and signal transduction were unveiled much later [2]. Protein methylation also affects packing of the side chain into binding pockets and is therefore thought to play a role in protein folding and protein–protein interactions [1]. While biochemists frequently study protein methylation and phosphorylation side-by-side, arginine methylation is generally believed to be irreversible and likely persist throughout the lifespan of a protein [2].

Three forms of arginine methylation have been identified in eukaryotes: ω-N^G-monomethylarginine (MMA), ω-N^G,N^G-dimethylarginine (asymmetric dimethylarginine, aDMA), and ω-N^G,$N^{G'}$-dimethylarginine (symmetric dimethylarginine, sDMA), all of which involve methylation of side-chain guanidino nitrogen atoms. ADMA is mainly located in non-histone nuclear proteins; MMA and sDMA are mostly found in myelin basic proteins [3]. Methylated arginines are released upon protein degradation. Through the action of nitric-oxide synthases, L-arginine is degraded into the vital signaling molecule nitric oxide [4]. Nitric oxide plays important roles in cardiovascular, nervous, and immune systems [5]. Both MMA and aDMA inhibit nitric-oxide synthases and block the production of nitric oxide [6]. As aDMA exists in much higher concentration than MMA, aDMA plays the major inhibitive role in nitric-oxide synthesis. Clinically, aDMA has been established as a

Abbreviations: Arg, arginine; MMA, monomethylarginine; aDMA, asymmetric dimethylarginine; sDMA, symmetric dimethylarginine; DDAH, dimethylarginine dimethylaminohydrolase; CID, collision-induced dissociation; QM, quantum mechanical.

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cardiovascular risk factor [5], indicator for metabolic syndrome [8] and renal dysfunction [7,9], and has been referred to as a uremic toxin [5]. Renal excretion and degradation by the enzyme N^G,N^G-dimethylarginine dimethylaminohydrolase (DDAH) are the two known clearance channels for aDMA [10]. Dysfunctions in renal excretion or the malfunction of the enzyme DDAH can both result in a detrimental rise in physiological aDMA levels so that nitric oxide generation is blocked and cardiovascular, neurological and other health threatening effects follow [6,11]. For sDMA, renal excretion has been the most significant clearance pathway known [11].

In terms of mass spectrometric detection, while Arg, MMA and DMA can be distinguished by their different molecular weights, the isomeric dimethylarginines (sDMA and aDMA) cannot be distinguished by mass measurement alone. MS/MS approach is required for distinguishing these isobaric DMAs. Since its introduction in 1968, collision-induced dissociation (CID) has played a vital role in the development of tandem mass spectrometry [12,13]. CID has found widespread applications in elucidating molecular structures and gas phase reaction mechanisms. In 1986, this method was first applied for finding the amino acid sequence information of peptides and proteins [14,15]. Several gas-phase fragmentation studies of arginine had been reported over the past two decades [16–19].

Methylation of the amino groups in the guanidino side chain increases the gas-phase basicity of the molecule and tends to localize the extra proton at the site of methylation in its pseudomolecular ion. In the CID study of methyl arginines carried out by Vishwanathan et al., some fragments could not be assigned unambiguously due to insufficient mass spectrometric performance [20]. Brame et al. investigated the effects of methylation on the fragmentation patterns of peptides containing arginine [21]. Here, we present the first high resolution CID studies that identifies many hitherto unreported isobaric fragments from the methylated arginines.

In its early development, MALDI was once considered unsuitable for small-molecule mass spectrometric analysis due to concomitant intense interfering matrix-originated chemical noise [22]. Donegan et al. changed this notion by admixing nitrocellulose with the MALDI matrix and effectively suppressed the matrix signals in the low molecular weight region of the MALDI mass spectrum [23]. Here we applied the nitrocellulose recipe of sample preparation for AP-MALDI and successfully generated high-quality MS data for the arginine derivatives of interest. Coupled with the superb performance of a 7.0 Tesla FTICR MS, we are able to elucidate a number of hitherto unreported isobaric molecular moieties and the fragmentation pathways of these ions.

2. Experimental

2.1. Chemicals and materials

Acetonitrile (HPLC grade), was obtained from J.T. Baker (USA). Deionized water was produced from distilled water with a UHQ water treatment system (Ultra Pure Water, ELGA, UK). The matrix α-cyano-4-hydroxy cinnamic acid (CHCA) was purchased from Aldrich (USA). Trifluoroacetic acid (TFA) was acquired from Acros Organics (New Jersey, USA). aDMA hydrochloride salt and sDMA sulfonate salt were purchased from Sigma (St. Louis, MO, USA). L-Arginine was obtained from Pierce (USA) and MMA from CN Biosciences (UK). Nitrocellulose was obtained from Schlicher & Schuell (Germany).

2.2. AP-MALDI sample probe preparation

Each commercial standard samples were dissolved in an aliquot of acetonitrile/water/TFA (50%/50%/0.1%) to a concentration of 40 pmol/μL and stored as stock solutions. CHCA and nitrocellulose stock solutions were separately prepared by dissolving weighed samples in acetone to a final concentration of 10 mg/mL and vortexed for 5 min. The matrix is prepared immediately before mixing with analyte, by mixing equal volumes of CHCA and nitrocellulose stock solutions and further diluted 5-fold with acetone and homogenized by vortexing for 5 min.

In studying the fragmentation patterns of the arginine derivatives, commercially acquired standard samples were used in generous quantities to facilitate MS/MS experiments. The loaded sample quantity decreased with its ionization efficiency, for example, 40 pmol of L-arginine, 20 pmol of MMA and 5–10 pmol of s/aDMA were consumed in preparing each individual MALDI target sample spot. In each case, equal volumes of the stock sample solution containing desired quantities of the sample amino acid and working matrix that contained 1 mg/mL each of CHCA and nitrocellulose, as described in the previous paragraph, were mixed on the MALDI sample plate. In a series of quick experiments with a single arginine derivative, the detection limits for Arg, MMA, and DMA were estimated to be roughly 5 pmol, 1 pmol, and 500 fmol, respectively.

2.3. AP-MALDI

All experiments were performed using a home-built AP-MALDI source coupled to a commercial FT ICR mass spectrometer operated at 7.0 T (Apex III, Bruker Daltonics, USA). The details of the hardware had been reported elsewhere [24], and only a brief description is given here. The UV laser was operated at its maximum repetition rate of 20 Hz triggered by the FTMS control system and synchronized with the data acquisition software such that the laser fires only during ion generation. Each spectrum resulted from summed accumulation of 10 scans acquired in the positive ion mode, and each scan was performed on ions generated from 50 laser shots that had been momentarily accumulated in an external linear ion trap and then collectively injected into the ICR cell for ensuing mass spectroscopic manipulations.

2.4. Mass calibration

The *Bruker* software *Data Analysis* was used for internal mass calibration and the smart formula function of the software was used for formulae prediction. All mass spectra were internally calibrated with the known fragment ions to accurately determine the masses of the unknown fragments. Sub-ppm mass accuracies were routinely achieved for all ionic species reported here.

2.5. On-resonance ion activation in CID experiments

The kinetic energy, in eV, of the ions trapped inside the infinity cell after on-resonance ion excitation can be calculated using the well-established equation [25–27]:

$$\text{K.E.}_{\text{post-excitation}} = \frac{\beta^2 q^2 V_{\text{p-p}}^2 (T_{\text{excite}})^2}{8d^2 m}$$

where q is the charge, $V_{\text{p-p}}$ is the excitation voltage, T_{excite} is the duration of the excitation waveform, d is the distance between the excitation plates (diameter of the cell), m is the mass of the molecule and β is the scaling factor. β for an infinity cell is ~0.9 [27,28]. All MS^2 and MS^3 experiments were carried out with a $V_{\text{p-p}}$ of 18 V and an excitation time (T_{excite}) of 50 μs. The diameter of the infinity cell is 0.06 m. As collision gas had been pulsed into the infinity cell while double resonance excitation occurred, the protonated arginine pseudomolecular ions underwent CID with a reciprocal mass-dependent maximum kinetic energy of 10.7–12.5 eV.

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