

Fragmentation of Singly Protonated Ions via Interaction with Metastable Rare Gas Atoms

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INTRODUCTION

A recently introduced novel fragmentation technique is based on interaction with metastable, electronically excited rare gas atoms. Fragmentation spectra of singly charged peptide ions were shown to be more structure-informative and very different from those obtained in low-energy collision-induced dissociation.¹ In this study we present results of in-depth investigation of the fragmentation mechanism.

METHODS

The experimental setup is described elsewhere.² Singly charged ions were produced in an ESI source and selected by a mass resolving quadrupole filter. Selected ions were trapped in the linear quadrupole ion trap for 20–100 ms and subjected to a flux of metastable rare gas atoms. The resulting product ions were analyzed in a time-of-flight mass analyzer.

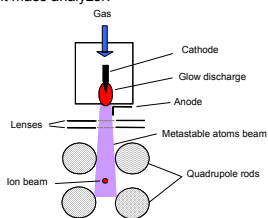


Figure 1. Schematic view of the fragmentation cell.

RESULTS

Figure 2A shows the CID spectrum of Fibrinopeptide A obtained using dipolar resonant excitation while Fig. 2B shows the fragmentation spectrum obtained via interaction with metastable helium atoms. The presence of a strong $[M+H]^{2+}$ ion signal in the spectrum indicates the formation of a radical odd-electron ion via Penning ionization of the singly protonated molecular peptide ion. The fragmentation pattern is dominated by a series of x-, y-, and z-ions, which result from a bond cleavage with the charge retained on the C-terminal fragment. Several of the x-type ions (denoted by *)

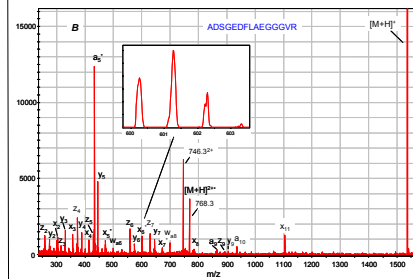
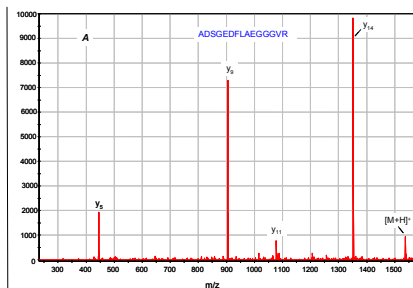


Figure 2.

reveal the presence of $(x+1)^+$ radical ion (see inset). It is an odd-electron ion, which forms through a homolytic radical cleavage of the α -carbon carbonyl-carbon bond. Several w-type fragment ions, which correspond to a side-chain loss, are also present in the spectrum.

The effect of the metastable level energy: 19.8 eV (He), 16.7 eV (Ne), 11.5 eV (Ar), 9.9 eV (Kr), and 8.3 eV (Xe) was studied by flowing different rare gases through the discharge source. Fragmentation spectra of Angiotensin II obtained via interaction with metastable helium and neon atoms were similar (spectrum for Ne is shown in Figure 3). The presence of a strong $[M+H]^{2+}$ ion signal indicates the formation (similarly to Fibrinopeptide A) of a radical odd-electron ion via Penning

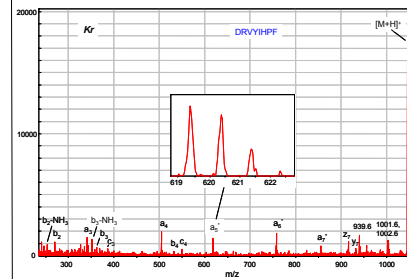
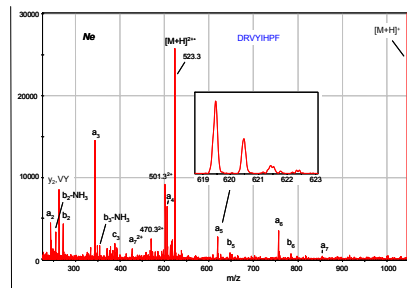


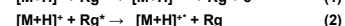
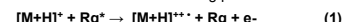
Figure 3. Fragmentation spectrum of singly charged Angiotensin II ions obtained using metastable Ne and Kr.

ionization. An intense doubly charged ion at $m/z = 501.3$ corresponds to a loss of 44 Da from $[M+H]^{2+}$ ion. A doubly charged ion at $m/z = 470.3$ corresponds to a loss of 106 Da. The fragmentation mass spectrum is dominated by a series of a- and b-ions, which result from a bond cleavage with the charge retained on the N-terminal fragment. The intensity of isotopic peaks for a-ions corresponds to an expected isotopic distribution (expanded view of a_5 ion isotopic distribution is shown in the inset).

The fragmentation spectrum was found to be different when Angiotensin II ions interacted with metastable krypton

atoms. The $[M+H]^{2+}$ ion and related ions with 44 Da and 106 Da mass loss are no longer observed. Instead, singly charged ions at $m/z = 939.6$ and 1001.6 are observed. These ions correspond to a neutral loss of 107 Da and 45 Da, respectively. The intensities of a- and b- fragment ions are considerably decreased. The expanded mass spectrum of a_5 ion (see inset) shows the intensity distribution, where the second isotope is more intense compared to that expected from normal isotopic distribution. This indicates the presence of $(a+1)^+$ radical odd-electron ion. Several c-ions are also observed in this spectrum. Fragmentation spectrum during interaction with argon metastable atoms was similar to krypton, with the exception of increased background noise in the low-mass region. When xenon was flowing through the discharge source, the number and intensity of observed a-fragments further decreased. Singly charged ions at $m/z = 939.6$ and 1001.6 were still observed.

Fragmentation mechanism. The energy transfer from the electronically excited metastable level to a singly charged peptide ion could lead to the following processes:



Ionization potentials of singly charged peptide ions lie in the range 10.5 – 11.5 eV. The metastable levels of He and Ne possess sufficient energy to initiate Penning ionization. The formation of a hydrogen-deficient peptide radical cation in process (1) is unique and was previously observed only through a low-energy CID of metal/peptide complexes. The formation of a radical cation in this process leads to a subsequent fragmentation. Because two charges are present, both fragments (corresponding to N- and C- termini) created during bond cleavage can retain a charge. This explains the presence of several a-type ions in fragmentation spectra of Fibrinopeptide A, which initially have a charge localized at C-terminus. The formation of doubly charged ions accompanied by neutral losses of 44 Da (CO_2 group) and 106 Da (C_6H_8O group from tyrosine in Angiotensin II) also confirms the internal excitation of doubly charged radical cations $[M+H]^{2+}$ formed via Penning ionization. Excitation of a peptide cation in process (2) can also lead to fragmentation since transferred energy exceeds the typical bond energy of 3–4 eV. In the case of Angiotensin II this process leads to production of $(a+1)^+$ radical ions, which are directly formed in the initial homolytic radical cleavage of the of the α -carbon carbonyl-carbon bond.

This novel fragmentation technique can also be used for other classes of molecules. The fragmentation spectrum of reserpine is shown in Figure 4:

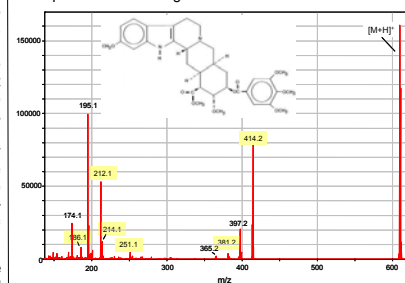


Figure 4. Fragmentation spectrum of reserpine obtained via interaction with metastable helium atoms.

Along with fragment ions identical to those observed in CID ($m/z = 174, 195, 365, 397$), additional ions (denoted by yellow boxes) appear in the spectrum. This allows obtaining data complementary to CID thus improving reliability of tandem MS analysis.

CONCLUSIONS

Fragmentation of singly charged ions via interaction with metastable atoms depends on metastable energy level and proceeds either via Penning ionization with formation of radical odd-electron doubly charged molecular cation or via high energy excitation of internal degrees of freedom.

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REFERENCES

- Berkout, V.D., Doroshenko, V.M., *Int. J. Mass Spectrom.* 278 (2008) 150-157.
- Berkout, V.D., *Anal. Chem.* 81 (2009) 725-731.

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