

# Fragmentation of Singly Charged Peptide Ions via Interaction with Metastable Atoms

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## INTRODUCTION

One of the limitations of tandem MS instruments, when using MALDI ionization for proteomics research, is the reduced fragmentation efficiency of singly charged peptide ions in low-energy CID. This effect is caused by a strong attachment of a proton to the C-terminus of a tryptic peptide which impedes the proton mobility along the peptide chain. As a result, fragmentation of singly charged tryptic peptides shows preferential cleavage at the C-terminal bond of acidic residues (i.e. aspartic and glutamic acid) and the N-terminal bond of proline. This preferential cleavage prevents the formation of a complete y-ion series, diminishing the informational content of the MS/MS spectra.

## METHODS

A time-of-flight (TOF) mass spectrometer with orthogonal acceleration, used in our experiments, is described elsewhere [1]. Singly charged peptide ions were produced in an ESI source from methanol/water solutions. Parent ions were selected by a mass resolving quadrupole filter. Selected peptide ions were trapped in the linear quadrupole ion trap for 20 - 100 ms and subjected to a flux of metastable rare gas atoms. A beam of metastable electronically excited atoms was produced in a glow-discharge source [1] and directed between the quadrupole rods.

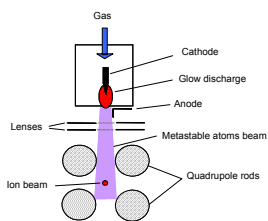


Figure 1. Schematic view of a source for the production of a metastable atoms beam.

The glow-discharge source has an asymmetrical electric field which allows an efficient separation of the neutral metastable atoms from charged particles. Interaction of the peptide ions, concentrated along the central axis of the quadrupole ion guide, with metastable atoms resulted in their fragmentation. Upon the ejection from the linear trap, product ions were analyzed in a time-of-flight mass spectrometer.

## RESULTS

To prove that the above source generates a predominantly neutral flow with the metastable atoms, the entrance capillary was closed and different gases were supplied into the mass resolving quadrupole section. This created a molecular flow of these gases to the linear trap. Mass spectra, averaged over 10 s, are shown below:

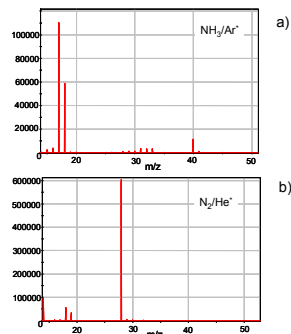


Figure 2. Ionization spectra of  $\text{NH}_3$  produced in interaction with metastable electronically excited argon atoms (a) and  $\text{N}_2$  - with electronically excited helium atoms (b).

The ionization potential of  $\text{N}_2$  is higher than the energy of the Ar metastable atoms. No ionization of  $\text{N}_2$  was observed with Ar in the discharge source (data not shown). The ionization potential of  $\text{NH}_3$  is about 1.5 eV lower than the Ar metastable levels. A strong molecular radical cation, produced via Penning

ionization, was detected when  $\text{NH}_3$  was supplied (Figure 2(a)). The energy of the He metastable states is greater than the nitrogen ionization potential. Strong  $\text{N}_2^+$  and  $\text{N}^+$  signals were observed when helium was introduced into the discharge source (Figure 2b). This indicates that the beam produced by the discharge source consists mainly of neutral particles and contains a substantial amount of metastable atoms.

With argon supplied into the discharge source, no fragmentation was observed for Bradykinin singly charged molecular ions (data not shown). The picture changed, when helium was used instead of argon:

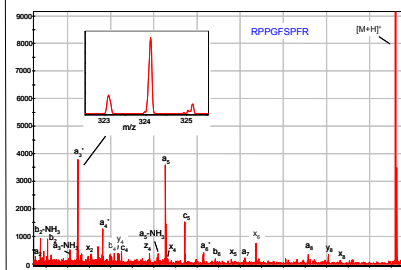


Figure 3. Fragmentation spectrum of singly charged Bradykinin ions obtained via interaction with metastable helium atoms.

The above fragmentation mass spectrum is dominated by a series of a-type ions, which result from a backbone cleavage between the  $\alpha$ -carbon and carbonyl carbon, with the charge retained on N-terminal fragments. Some weak x-type ions, which result from the same bond cleavage, where the charge is retained on the C-terminal, are also observed. Several of the a-type ions (denoted by \*) reveal the presence of (a+1)- radical ion (see an inset). Some y-, b-, c-, and z-type fragments are also observed.

Fragmentation spectrum of a singly charged Fibrinopeptide A and its synthetic analog reveals that fragmentation proceeds via energy transfer:

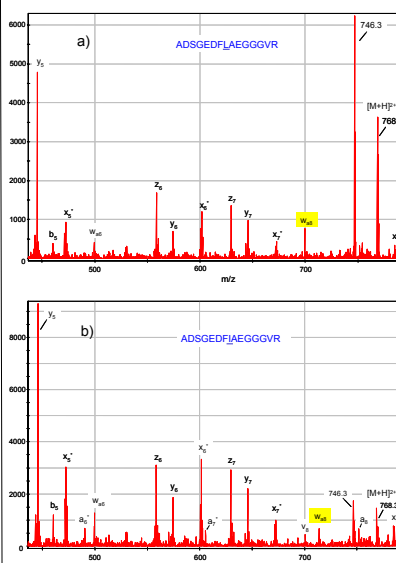


Figure 4. Fragmentation spectrum of singly charged Fibrinopeptide A ions (a) and its synthetic analog with Leu replaced by Ile (b) obtained via interaction with metastable helium atoms.

A strong  $[\text{M}+\text{H}]^{2+}$  ion signal, formed via Penning ionization of the  $[\text{M}+\text{H}]^+$  ions, is observed in this case. Mainly x-, y- and z-type ions (charge retained on C-terminal) are observed. The formation of w-type ions, which correspond to a side-chain loss, allows differentiation between Leu and Ile.

Fragmentation spectrum of a singly charged Substance P also shows a strong  $[\text{M}+\text{H}]^{2+}$  ion signal (Figure 5). A nearly complete series of a-type fragment ions is observed, which

is consistent with charge retention on the N-terminal (where arginine is located). No radical (a+1)- ions are recorded. Also observed are d- type fragments corresponding to a side-chain loss from a- type ions. Several b- and c-type fragment ions are also present in this spectrum.

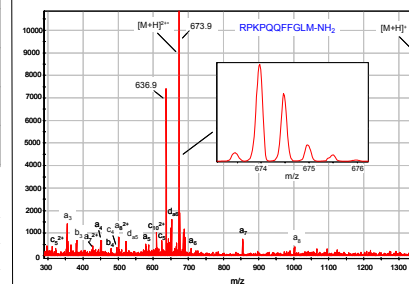


Figure 5. Fragmentation spectrum of singly charged Substance P ions obtained via interaction with metastable helium atoms.

## CONCLUSIONS

Fragmentation of singly charged peptide ions via interaction with metastable atoms was demonstrated for the first time.

Fragmentation channels include side-chain losses allowing differentiation between Leu and Ile residues.

## ACKNOWLEDGEMENTS

The author gratefully acknowledges Prof. Peter O'Connor for helpful discussions and the National Institutes of Health for their financial support (SBIR grant 2R44RR022926-02).

## REFERENCES

- Berkout, V.D., Fragmentation of protonated peptide ions via interaction with metastable atoms, *Anal. Chem.* 78 (2006) 3055-3061.