ECD and EID of Amyloid Beta and Synthetic β-Peptides.

Nadezda P. Sargaeva[1], Chunxiang Yao[1], Tzu-Yung Lin[1], Weidong Cui[1], Konstantin Aizikov[1], Xiaojuan Li[1], Cheng Lin[1], Peter B. O'Connor[1,2]

[1]: Boston University School of Medicine, Boston, MA

[2]: Department of Chemistry, University of Warwick, Coventry, United Kingdom

Introduction

Aspartic acid is isomerized to isoaspartic acid (at 1, 7, and 23 residues) in Amyloid beta protein fragments found in the brain of Alzheimer's patients. Electron Capture dissociation and Electron Ionization dissociation methods were applied to study the isomerization event. We compare ECD of 17-28 fragment and its isoaspartic version, and further analyze the isomerized one with EID technique. ECD was also applied to entire Amyloid beta protein fragments 1-40 and 1-42.

Isomerized aspartic acid is a β-linked amino acid. Therefore, its distinct fragmentation may be similar in β-peptides. To study fragmentation pattern of β-linked peptides Substance P was synthesized with the modification of 2 amino acid residues into β-amino acids (R-P-K-P-βQ-Q-F-F-G-βL-M-NH2) and ECD was applied.

Methods

All experiments were performed on a custom built qQq-FTICRMS with a nanospray source and 7T actively shielded magnet. Ions isolated in Q1 were accumulated in Q2 and transmitted into the ICR cell where they were irradiated with electrons of various energies emitted from a dispenser cathode (0-40eV). Proteins and peptides were electrosprayed with 10^{-5} M concentrations in 50:50 methanol water with 1% formic acid.

Results

ECD of synthesized Amyloid beta protein fragment 17-28 with isoaspartic acid at the 23^{d} position shows distinct z-57/c+57 fragmentation pattern, which could be used to identify the presence of isoaspartic acid. EID of this fragment demonstrates highly abundant fragmentation including all types of fragments (fig.1). Characteristic to IsoAsp, the $z^{\bullet}_{6}$-57 fragment is also present in the spectra. The abundance of this peak is low compared to ECD, as all of the *c*- and *z*- type of fragments are; nevertheless, this experiment demonstrates that EID can be applied in isoaspartomics research alternatively to ECD. This could be essential in the case of singly charged ions where ECD is not applicable.

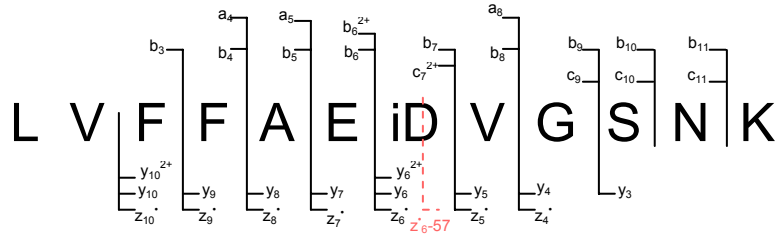

Figure 1. Fragments observed in EID of Amyloid beta protein fragment 17-28 isomerized Asp23.

ECD of Amyloid beta protein fragments 1-40 and 1-42 showed 75% of sequence coverage (fig. 2). Importantly, many fragments next to Asp and Asn residues were detected. We expect similar behavior for IsoAsp containing peptides, which could be used to determine the presence of isoaspartic acid and deamidation when it occurs.

Because only singly charged ions were observed, the small β-peptide $V_{\beta 2}A_{\beta 2}L_{\beta 2}V_{\beta 3}A_{\beta 3}L_{\beta 3}$ was analyzed by EID technique. Only *b*- and *y*- types of fragments were generated. The ECD spectrum of β-substance P is different from that of non-modified sub P. Similar to IsoAsp fragmentation we expected to see the signature fragments c^{+}*[side chain],* $z^{\bullet}$*-[side chain]* in the spectrum. Surprisingly, we observed no such fragments. Additionally, c_4 and c_9 fragments adjacent to modified acids were absent. This is probably due to lack of the stabilization effect of the carbonyl group as in the case of IsoAsp containing peptides.

Conclusions

Amyloid beta can be analyzed using ECD and EID for detection of isomerized aspartic acid residues. Unlike β-aspartic acid, other β-linked amino acids appear to suppress *c-/z-* type fragmentation at these positions, an observation which can be used (with appropriate standards) to detect them and determine their positions.

Novel aspect

ECD and EID were used to study Amyloid beta protein fragments and β-peptides.

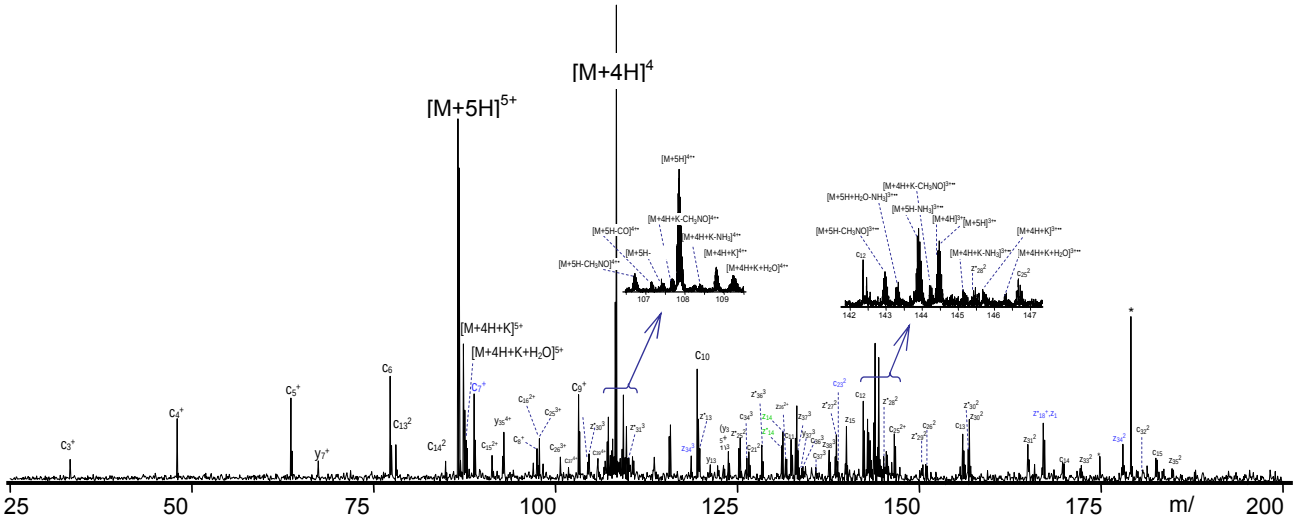

Figure 2. ECD of Amyloid beta protein fragment 1-40.