Challenges in identification of an N-terminal isoaspartic acid residue.

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Introduction: Formation of isoaspartic acid (isoAsp) is a common modification of aspartic acid (Asp) or asparagine (Asn). Differentiation of isoAsp/Asp isomeric residues is a challenging task owing to their similar properties and identical molecular mass. However, they can be differentiated using ion-electron/ion-ion interaction fragmentations (ExD), as these methods provide diagnostic fragments *c + 57* and $z^{\bullet}$ - 57 specific to the isoAsp residue. To date, however, the presence of diagnostic fragments has not been explored on peptides with N-terminal isoAsp residue. To address this question, various peptides containing N-terminal isoAsp were analyzed in this study, including the Angiotensin II (AngII) peptide variants D(iD)RVYIHPF, synthetic peptide variants Ac-D(iD)GVGD(iD)VGGVH-NH_2, and Amyloid beta 1-10 (A_β10) peptide variants D(iD)AEFRHD(iD)SGY.

Methods: The A_β10 peptide variant (NAEFRHNSGY) was deamidated at pH 7.7 at 37 °C for 4 days. The resulting mixture of A_β10 isomers was separated on C18 column in a linear gradient of acetonitrile with 0.1% formic acid (FA) using reversed-phase high performance liquid chromatography (RP-HPLC) Agilent 1200 Series system (Agilent Technologies). Fractions were collected for off-line MS/MS analysis.

ExD analyses were performed on SolariX FTICR (Bruker Daltonics), LTQ-Orbitrap XL (Thermo Scientific), and AmaZon Ion Trap (Bruker Daltonics) instruments. Peptides were nanosprayed or electrosprayed with 0.5-2 uM concentrations in mobile phase solution or in 50:50 methanol:water with 0.1% FA when infused directly.

Preliminary data: Signature fragments $z_n^{\bullet}$*-57* (appeared as $M^{\bullet}$ *-74* or $M^{\bullet}$ *-116* when N-terminus was acetylated) were observed for N-terminal isoAsp residues using ExD methods. A mixture of nine peptides, produced upon deamidation of the A_β10 peptide variant (**N(D)(iD)**AEFRH**N(D)(iD)**SGY), was separated and each peptide identified using combined approach of off-line RP-HPLC and ECD-FTICRMS.

It should be noted that the signature fragment ion detection was challenging as the peak intensity was low for all peptides. In particular, in ETD (Orbitrap), the peak corresponding to the signature fragment ion was barely observable above the noise threshold level. ECD provided the highest S/N ratio for the signature fragment ions studied, possibly owing to the higher energy available in the ECD process than in the ETD process.

The effect of additional energy on the signature fragment ion intensity was further investigated by means of supplemental activation (ETD on Orbitrap), hotECD (ECD on SolariX), and smart decomposition (ETD on AmaZon). Apparently, the results depended on the peptide sequence, specifically, the presence of basic residues and side chain interactions. In some cases, the S/N ratio of the signature fragment ion peak absolute intensity was increased by up to 6 times.

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Novel aspect: N-terminal isoAsp residues were identified using ExD methods in various peptides including synthetic Amyloid$_\beta$(1-10) by detecting signature fragment ions $z_n^{\bullet}$*-57*.