In-depth characterization of protein-based therapeutics using collision-induced dissociation (CID) and electron-activated dissociation (EAD)

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Background

MS-based peptide mapping is routinely used to monitor product/critical quality attributes of protein therapeutics, such as oxidation, deamidation and glycosylation. To generate a peptide map, proteins are often digested with trypsin and peptides are identified via data-dependent acquisition (DDA) using collision-induced dissociation (CID). Since trypsin cleaves at the C-termini of arginine (R) and lysine (K), proteins with infrequent R and K generate longer peptides, which are poorly fragmented using CID. Complete sequence coverage of these proteins necessitates the use of multiple proteolytic enzymes, which complicates the sample preparation procedure and MS data interpretation. Here, we evaluate a tunable electron-activated dissociation (EAD) approach for the fragmentation of long tryptic peptides and differentiation of deamidation isomers, namely, aspartic acid (Asp) and isoaspartic acid (isoAsp).

Materials and Methods

Sample preparation

Two proprietary proteins (P1 and P2) were digested with trypsin, followed by reduction and alkylation.

Chromatography

The digested samples were separated with an ACQUITY CSH C18 column (Waters) using an ExionLC AD system (SCIEX).

Mass spectrometry

LC-MS data were acquired with DDA using either CID or EAD on a ZenoTOF 7600 system (SCIEX). Data analysis was performed using Biologics Explorer software (SCIEX).

ZenoTOF 7600 system

Results

Figure 1. CID vs. EAD of a long peptide from P1

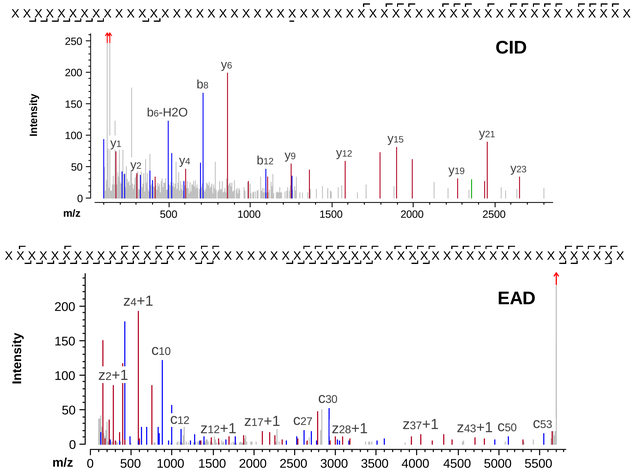
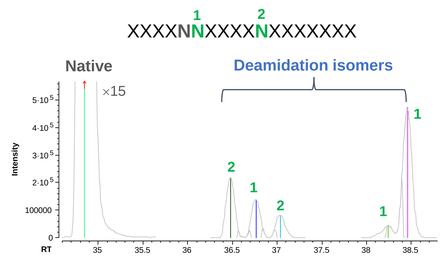


Table 1. Number of cleavages measured for long peptides A and B using CID vs. EAD

Protein	Peptide	Length	Charge State	# cleavage (CID)	# cleavage (EAD)
P1	A	55	5+ - 7+	29	45
P2	B	52	6+	16	34

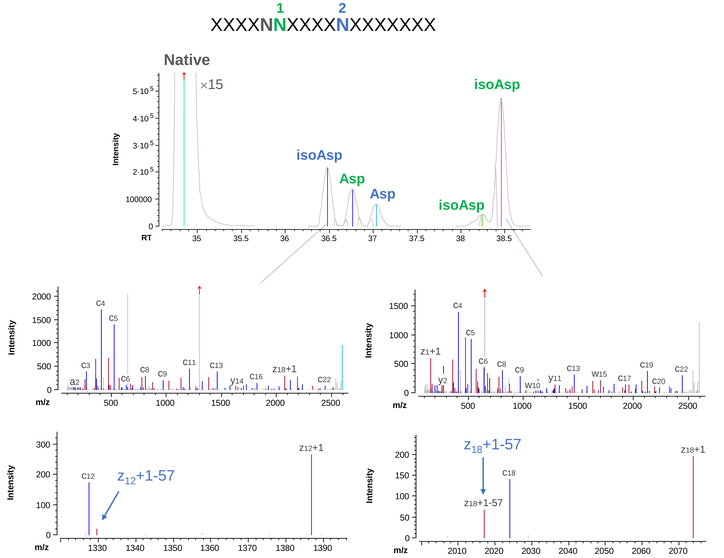
- EAD led to more extensive fragmentation of long peptides than CID
- EAD provided a much higher sequence coverage of 2 long peptides for improved confidence in peptide identification and sequence analysis

Figure 2. Detection of 5 deamidation isomers of a peptide from P2



- Deamidation of Asn1 and Asn2 led to the formation of 3 and 2 Asp/isoAsp isomers, respectively

Figure 3. Differentiation of deamidation isomers using EAD



- Elution order alone is not completely reliable for the differentiation of Asp vs. isoAsp isomers
- Confident differentiation of Asp vs. isoAsp was achieved based on the detection of diagnostic z-57 or c+57 (not shown) ions for isoAsp

Conclusion

- Long peptides are commonly observed in trypsin digestion of protein-based therapeutics due to a lack of Arg or Lys residues in certain parts of the sequence
- Compared to the traditional CID approach, EAD provided more extensive fragmentation of long peptides, thereby increasing the confidence in their identification and sequence analysis
- The ability of EAD to differentiate amino acid isomers, such as Asp and isoAsp, was leveraged to elucidate a complex deamidation profile observed for a P2 peptide

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