A single-injection workflow for enhanced peptide mapping using collision-induced dissociation (CID) and electron activated dissociation (EAD)

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ABSTRACT

This poster describes a single-injection CID/EAD peptide mapping workflow for the enhanced characterization of biotherapeutics. This workflow combines the advantages of CID and EAD in 1 method with minimal adjustment required for the existing CID platform method. This workflow provies high sequence coverage (>96%) of light and heavy chains (LC and HC) in a single experiment and confident identification of singly charged or long peptides. The workflow enables the unambiguous differentiation of amino acid isomers such as aspartic (Asp) vs. isoaspartic (isoAsp) acids and the accurate localization of labile post-translational modifications (PTMs), such as N- and O-linked glycosylation.

INTRODUCTION

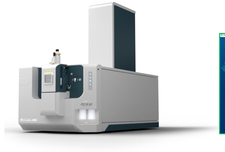
Peptide mapping is widely used for sequence confirmation and PTM identification for antibody-based therapeutics. Peptide mapping is typically performed with collision-based MS/MS fragmentation methods, such as CID. While CID offers high sensitivity and efficient fragmentation of common peptides, it is limited in its abilities to fragment long peptides, localize labile PTMs and differentiate amino acid or positional isomers. By comparison, electron-based MS/MS approaches, such as EAD, offer excellent fragmentation of long peptides, accurate localization of labile PTMs and confident isomer differentiation.[1-4] In this work, a joint CID/EAD method was developed to provide enhanced peptide mapping results in a single injection. The CID/EAD workflow combines the advantages of these 2 MS/MS techniques with minimal adjustments to the existing CID platform method.

MATERIALS AND METHODS

Sample:
NISTmAb and etanercept were denatured by guanidine hydrochloride, reduced with dithiothreitol, and alkylated using iodoacetamide. The samples were then buffer exchanged using Bio-Spin columns (Bio-Rad) and digested using trypsin/Lys-C mix (Promega). The etanercept digest was further treated with SialEXO (Genovis) to remove sialic acids.

HPLC:
The peptides were chromatographically separated with a 60-minute LC gradient using an ACQUITY CSH C18 column (Waters) at a flow rate of 0.25 mL/min. The column was kept at 60°C in the column oven of an ExionLC AD system (SCIEX).

Mass spectrometry:
LC-MS data were acquired with CID and EAD data-dependent acquisition (DDA) in SCIEX OS software (SCIEX) using the ZenoTOF 7600 system (SCIEX). The CID/EAD DDA method was built by combining CID and EAD DDA in the same method with minor adjustments of the number of precursors and accumulation time for MS/MS. DDA data were analyzed using the pre-built peptide mapping workflow templates in Biologics Explorer software.

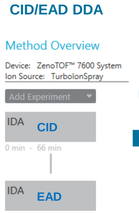


ZenoTOF 7600 system with Zeno CID and Zeno EAD

Biologics Explorer software with pre-built peptide mapping workflow templates

RESULTS

CID/EAD DDA



Single injection

Sequence coverage map

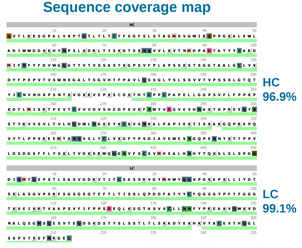


Figure 1. The joint CID/EAD DDA method offers high sequence coverage (>96%) of NISTmAb LC and HC in a single injection of a trypsin digest. The CID/EAD workflow combines CID and EAD in the same method. This method provides comparable or better sequence coverages of LC and HC compared to the method using CID or EAD alone.

Characterization of short and long peptides

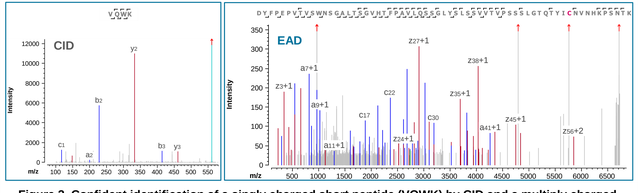


Figure 2. Confident identification of a singly charged short peptide (VQWK) by CID and a multiply charged long peptide (~6.7kDa) using EAD. The 2 complementary fragmentation techniques offered by the CID/EAD method allowed confident identification of challenging peptides, such as singly charged short peptides or multiply charged long peptides. The short and long peptides shown here are from LC and HC of NISTmAb, respectively.

Differentiation of Leu vs. Ile residues

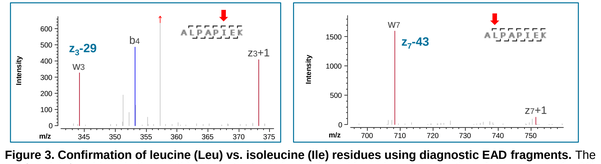


Figure 3. Confirmation of leucine (Leu) vs. isoleucine (Ile) residues using diagnostic EAD fragments. The Leu[2] and Ile[6] residues in the peptide ALPAPIEK can be confirmed based on the detection of signature w_3 (z_3-29) and w_7 ions (z_7-43) for Ile and Leu, respectively, in the EAD data.

Differentiation of Asp vs isoAsp residues

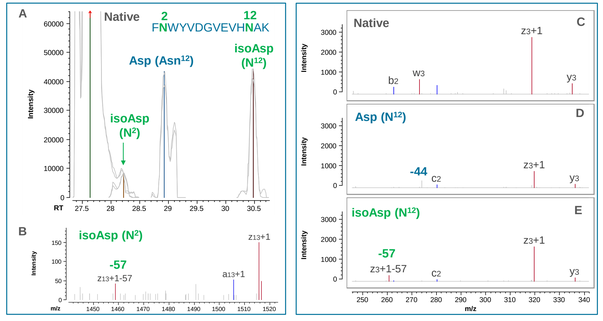


Figure 4. Identification and differentiation of Asp vs. isoAsp isomers using EAD. Three deamidation isomers were identified in the extracted ion chromatogram of peptide FNWYVDGYEVHNAK (A). EAD led to the differentiation of these 3 isomers based on the detection of a z-57 or z-44 fragment (B-E). The detection of a z_{13}-57 ion (B) confirms the isoAsp isomer from N^2 deamidation (B), while the detection of a z_3-44 or z_3-57 fragment enabled the differentiation of Asp vs. isoAsp isomers from N^{12} deamidation (D and E).

Localization of N-linked glycosylation

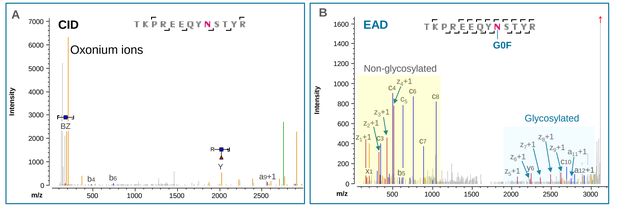


Figure 5. Characterization of N-linked glycosylation in NISTmAb using CID and EAD. The CID/EAD workflow provided complementary CID and EAD data for confident identification and localization of N-linked glycosylation. While CID generated abundant oxonium ions to confirm the presence of glycosylation in peptide TKPREEQYNSTYR (A), the EAD spectrum of this peptide (B) provides with a nearly complete series of c/z ions with (shaded in blue) or without (shaded in yellow) glycosylation. Specifically, the detection of non-glycosylated z_1-z_4 and glycosylated z_5-z_9 fragments allows accurate localization of the G0F glycan to the Asn residue.

Localization of O-linked glycosylation

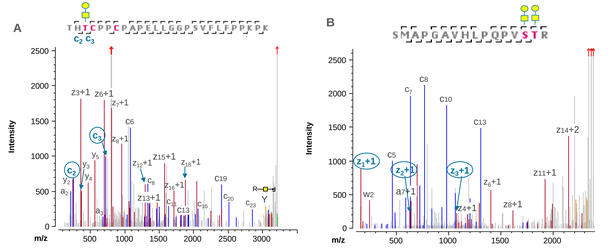


Figure 6. Accurate localization of O-linked glycosylation in etanercept using EAD. The location of 1 HexHexNAc moiety in the glycopeptide THTCPPCPAPELLGGPSVFLFPPKPK was pinpointed to the Thr[3] residue out of 3 potential O-linked glycosylation sites including 2 Thr and 1 Ser based on the detection of non-glycosylated c_2 and glycosylated c_3 fragments (circled in A). Similarly, the sites of 2 O-glycans in the glycopeptide SMAPGAVHLPQPVSTR were confidently determined based on the detection of z_1-z_3 (circled in B) with 0, 1 and 2 HexHexNAc moieties, respectively.

CONCLUSIONS

- The single-injection CID/EAD workflow combines the advantages of complementary CID and EAD techniques and offers a comprehensive characterization of biotherapeutics.
- The CID/EAD workflow offered high sequence coverage (>96%) of LC and HC from 1 trypsin digest of NISTmAb in a single injection.
- While CID provided confident identification of short peptides, EAD led to excellent fragmentation of long peptides.
- EAD data provided by the CID/EAD workflow enabled confident differentiation of amino acid isomers such as Leu vs. Ile and Asp vs. isoAsp and accurate localization of N- and O-linked glycosylation.
- The CID/EAD workflow requires minimal adjustment to the existing CID platform method while offering additional benefits for isomer differentiation and PTM analysis using EAD.

REFERENCES

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2. Comprehensive characterization of O-linked glycosylation in etanercept by electron activated dissociation (EAD). SCIEX technical note, RUO-MKT-02-14921-A.
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4. Monitoring and determining the cause of antibody discoloration using capillary isoelectric focusing (cIEF) and electron activated dissociation (EAD). SCIEX technical note, MKT-26819-A.

TRADEMARKS/LICENSING