A novel fragmentation technology allows for in-depth glycopeptide characterization in glycoproteins

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INTRODUCTION

A newly developed electron activated dissociation (EAD) approach allows to tune electron energy to produce different fragmentation patterns of the electron-based dissociation (ExD) family.
The data presented here demonstrate the advantage for glycoproteins using this novel fragmentation cell over traditional collision-induced dissociation (CID) used for peptide mapping, which does not allow for consistent identification and localization of glycans on peptides.
Combining increased detection of fragments using a mechanism for increased duty cycle on a Q-TOF instrument (the Zeno trap) enables higher confidence in data assignment, making the Zeno EAD combination ideal for in-depth analysis of glycopeptides.

Key features of the SCIEX solution

- EAD allows in combination with fast data-dependent acquisition (DDA) greater in-depth analyses of next generation glycoprotein therapeutics and standard mAbs by offering an alternative fragmentation
- The tunable electron energy provides a higher level of structural information for glycopeptide characterization. It resulted in rich fragmentation with predominantly peptide backbone fragments leaving the glycan intact on the glycosylation site, enabling localization information.
- Increased detection of fragments (5 to 10-fold) was achieved using the Zeno trap. Enhanced sensitivity is required with alternative fragmentation compared to CID, due to the greater number of diagnostic fragments created.
- Reproducible fragmentation with EAD for singly, doubly and multiply charged ions. EAD is capable of fragmenting peptides with a variety of charge states.
- Fully automated DDA using EAD with SCIEX OS software and automated data interpretation with the new Biologics Explorer software from SCIEX offer a streamlined characterization workflow

MATERIALS AND METHODS

Sample preparation:
Therapeutic monoclonal antibodies adalimumab, rituximab as well as an antibody-drug-conjugated sample (ado- trastuzumab emtansine) were denaturated with 7.2 M guanidine hydrochloride, 100mM Tris buffer (pH 7.2), followed by reduction with 10mM DL-dithiothreitol and alkylation with 30mM iodoacetamide. Digestion was performed with trypsin/Lys-C enzyme at 37ºC for 16 h.

Time [min]	Mobile phase A [%]	Mobile phase B [%]
Initial	98	2
5	98	2
6	90	10
40	55	45
44	10	90
46	10	90
47	98	2
50	98	2
51	10	90
54	10	90
55	98	2
60	98	2

HPLC conditions:
An ExionLC system equipped with a CSH C18 column (1.7 µm particle size, 130 Å, 2.1×100 mm, Waters) was used to separate 10 µL (4 µg) of the trypsin/Lys-C digest. The mobile phase A consisted of water with 0.1% formic acid, while the organic phase B was acetonitrile 0.1% formic acid. A gradient profile was used at a flow rate of 300 µL/min. The column temperature was maintained at 50ºC.

Parameter	CID	EAD
Collision energy	*rolling*	*12 V*
Electron KE	*NA*	*7 eV*
Electron beam current	*NA*	*5500 nA*
ETC	*NA*	*100*
Zeno trap	*ON*	*ON*
Accumulation time	*0.05 s*	*0.09 s*

MS/MS conditions:
Data were acquired using DDA on a QTOF system (ZenoTOF 7600 system, SCIEX) with either CID or EAD, a novel type of ExD.[1-4]

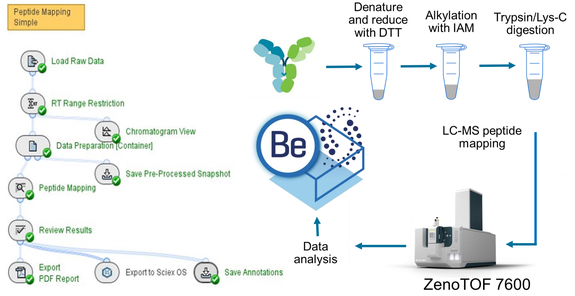

Figure 1. Automated typical "Peptide Mapping Simple" workflow in Biologics Explorer software used for N-glycopeptide analysis of CID and EAD data sets.

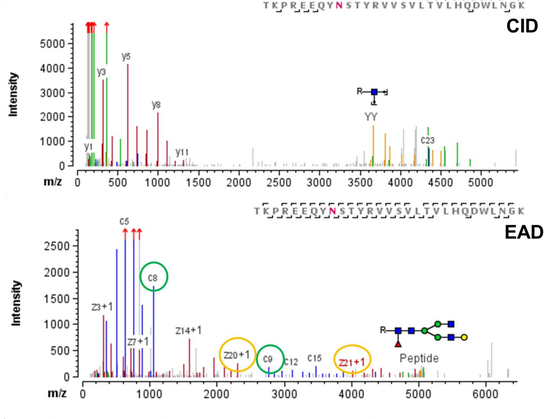

Figure 2. Comparison of MS/MS data from a glycopeptide containing G1F using CID and EAD. Top panel shows CID data, while bottom shows EAD data. Circled in green are indicative *c*-ions (*c*8, *c*9) for the glycan while circled in orange are the indicative *z*-ions (*z*20, *z*21). Blue (*c*- and *b*-ions) and red (*z*- and *y*- ions) deisotoped, deconvoluted and centroided peaks indicate the ions identified. In addition, diagnostic fragment ions confirm the localization of the glycosylation (encircled ions) in the case of EAD; whereas CID did not provide this information. In the CID spectrum red peaks show peptide fragments (y-ions) and orange peaks show oxonium ions of glycan portion.

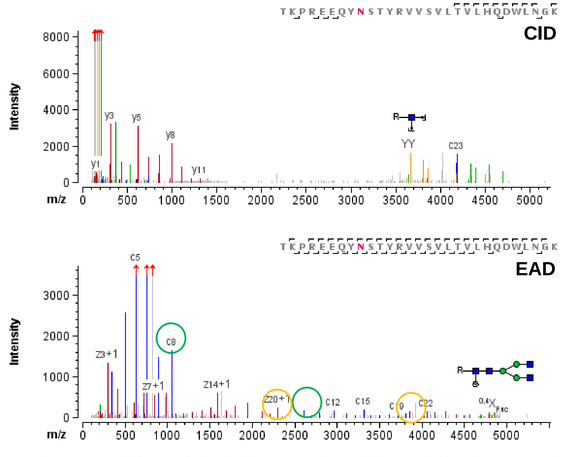

Figure 3. Comparison of CID and EAD MS/MS data for glycopeptide with G0F. Top: CID did not provide descriptive fragment information, neither positional information for the glycosylation. Bottom: close to 100% fragment coverage was achieved with EAD and diagnostic fragment ions confirm the localization of the glycosylation (encircled ions *c*8/*c*9 and *z*20/*z*21).

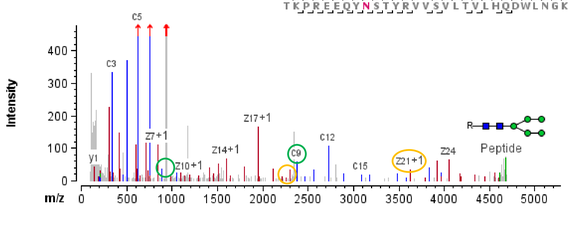

Figure 4. EAD MS/MS spectrum for glycopeptide with Man5. MS/MS spectrum is shown for a low abundance glycopeptide (*z* =+5) of ~3% relative abundance. EAD achieved extensive fragment coverage and localization of the glycosylation site. Diagnostic fragments *c*8/*c*9 and *z*20/*z*21 are circled in the spectrum.

Modifi…	20210130 Herceptin IDA ECD …	20210130 Herceptin IDA ECD …	20210130 Herceptin IDA ECD Most intense _3 [%]
G0	3.23	3.20	3.13
G0-GlcNAc	0.57	0.58	0.60
G0F	46.08	46.09	45.48
G0F-GlcNAc	6.03	6.26	6.42
G1	1.08	1.07	1.03
G1F	34.88	34.60	35.17
G2F	4.36	4.32	4.41
Man5	3.27	3.35	3.24
Man6	0.42	0.43	0.40

Figure 5. Identification summary of N-linked glycosylations in trastuzumab at N300. The table from the Biologics Explorer software summarizes the identified glycan species based on MS/MS with EAD and the relative abundance based on the XIC of the MS1 for 3 replicate injections. G0F and G1F are the most abundant glycoforms observed. Trace level of high mannose were also detected. The table confirms high reproducibility from high-abundance modified peptides to very low-abundance forms.

CONCLUSIONS

- The robust, reproducible and easy-to-use alternative fragmentation mechanism of EAD enables users to identify, fully characterize and relatively quantify glycopeptides along with a general peptide mapping analysis in 1 injection
- Excellent fragment coverage and localization of fragile modifications can be achieved with Zeno EAD with very high reproducibility, allowing for full confidence in peptide ID
- MS/MS fragment detection can be significantly enhanced compared to traditional MS/MS analyses, enabling collection of high-quality data for confident fragment assignment utilizing Zeno EAD [4-5]
- Automatic data processing enables the routine and advanced characterization of complex biotherapeutics and standard mAbs in a reproducible manner using the new Biologics Explorer software from SCIEX [6]

REFERENCES

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