Comparison of ECD and UVPD MS/MS for the Relative Quantitation of the Isomeric Products of Deamidation

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Introduction

- Deamidation of asparagine (Asn) is a spontaneous and non-enzymatic post translational modification in proteins, playing a significant role in ageing diseases such as Alzheimer's and Parkinson's disease.[1]
- RP-HPLC can be used for separation of the isoD and D (products of Asn deamidation) but it can be difficult to achieve baseline separation.
- Cleavage of the C_α - C_β bonds in the peptide backbone by electron capture dissociation tandem mass spectrometry (ECD MS/MS) generates diagnostic fragment ions for isoAsp residues, c•r + 58 and z_{l-r} -57.[2]
- Ultraviolet photodissociation (UVPD) is a higher energy activation method, shown to result in extensive fragmentation of peptides and proteins[3] and this method will be utilised for quantitation of the isomers.

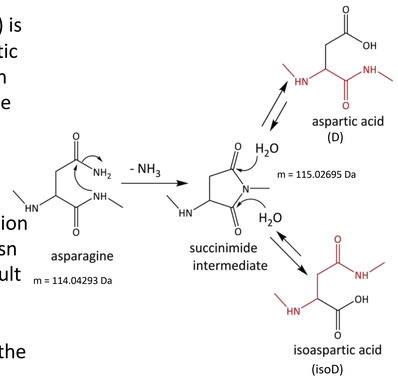


Figure 1. Mechanism for deamidation of Asn and isomerization of D to isoD via hydrolysis of the succinimide intermediate.[1]

Experimental Setup

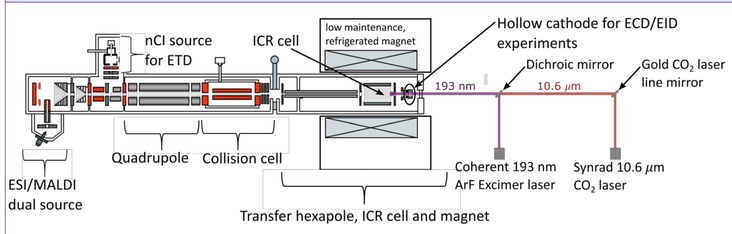


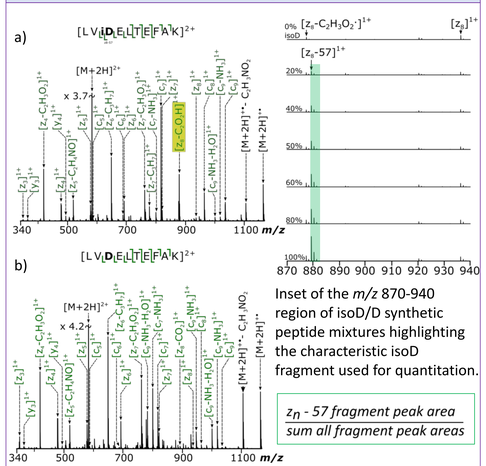
Figure 2. Schematic representation of the Bruker 12T SolariX FT-ICR mass spectrometer (courtesy of Bruker Daltonik, Bremen, Germany) equipped with a 193 nm ArF excimer laser for UVPD and a CO_2 laser for infrared multiphoton dissociation.

Methods

Samples: Deamidated and trypsin digested bovine serum albumin (BSA) (Sigma Aldrich) and synthetic peptides (Genscript) were diluted with water: acetonitrile (50:50, v/v) with 0.1% formic acid into final concentrations of 1 – 5 uM.
MS and ECD MS/MS: Bruker 12T SolariX FT-ICR MS; sprayed by nano ESI (positive ion mode) with ECD MS/MS. Electrons were emitted from an indirectly heated hollow dispenser cathode held at 1.5 A.
UVPD: ExciStar ArF 193 nm laser (10 Hz, Coherent, UK) with data acquired with 1 shot at 5 mJ/pulse.

Results and Discussion

ECD MS/MS

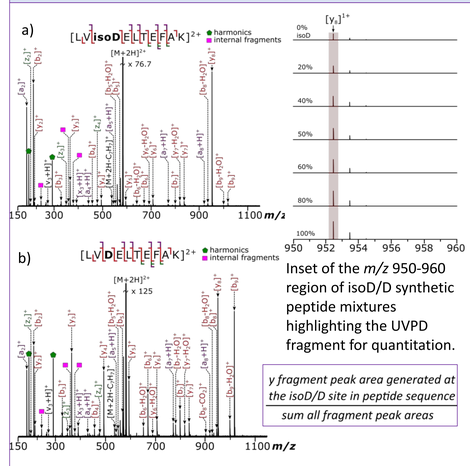


Inset of the *m/z* 870-940 region of isoD/D synthetic peptide mixtures highlighting the characteristic isoD fragment used for quantitation.

$$\frac{z_n - 57 \text{ fragment peak area}}{\text{sum all fragment peak areas}}$$

Figure 3. ECD MS/MS fragmentation spectra of a) LVisoDELTEFAK and b) LVDELTEFAK (absolute average mass error < 0.4 ppm).

UVPD MS/MS



Inset of the *m/z* 950-960 region of isoD/D synthetic peptide mixtures highlighting the UVPD fragment for quantitation.

$$\frac{y \text{ fragment peak area generated at the isoD/D site in peptide sequence}}{\text{sum all fragment peak areas}}$$

Figure 5. UVPD MS/MS fragmentation spectra of a) LVisoDELTEFAK and b) LVDELTEFAK (absolute average mass error < 0.4 ppm).

Calibration Curves

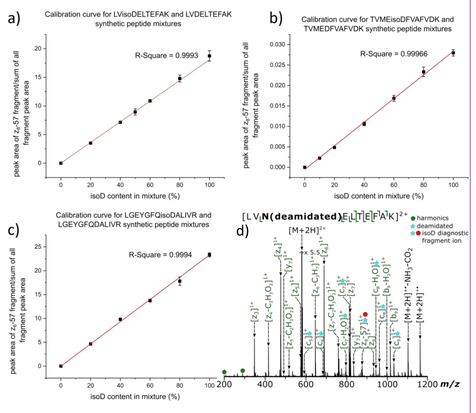


Figure 4. Linear calibration curves a)-c) generated for three different isoD/D synthetic peptide mixtures (R^2>0.99) and d) ECD MS/MS spectrum of the deamidated peptide from the bovine serum albumin (BSA) tryptic digest (incubated in ammonium bicarbonate (10 mM) (pH 8.0) at 60 °C for 5 days).

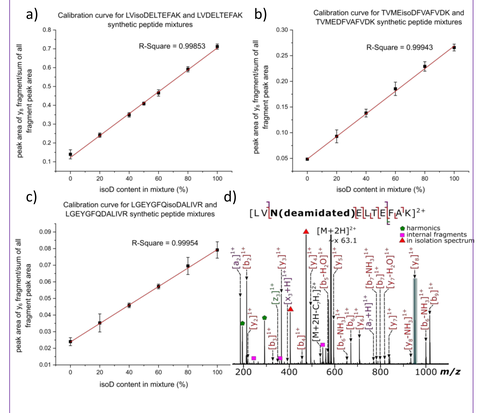


Figure 6. Linear calibration curves a)-c) generated for three different isoD/D synthetic peptide mixtures (R^2>0.99) and d) UVPD MS/MS spectrum of the deamidated peptide from the bovine serum albumin (BSA) tryptic digest (incubated in ammonium bicarbonate (10 mM) (pH 8.0) at 60 °C for 5 days).

Table 1: Percentage isoD determined in deamidated BSA peptides using ECD MS/MS and UVPD MS/MS.

Peptide	% isoD from ECD MS/MS results	% isoD from UVPD MS/MS results
LVN(Deamidated)ELTEFAK	35.9 ± 0.2	41.8 ± 0.2
TVMEN(Deamidated)FVAFDK	13.2 ± 0.5	17.4 ± 0.3
LGEYGFQN(Deamidated)ALIVR	72.2 ± 0.4	69.4 ± 0.6

- Calibration curves were obtained with data obtained using both methods with good linearity (R^2>0.99) for all peptides studied.
- The intensity of the b/y ions generated at the specific isoD or D position in the peptide sequence by UVPD can be used to discriminate between the isomeric peptides due to isoD peptides containing higher y-ion intensities at the deamidated sites compared to the D peptides as shown in Figure 5.
- The results show that the percentage isoaspartic acid determined using the ECD MS/MS and UVPD MS/MS are comparable (within a maximum of 6% difference for the isoaspartic acid percentage content between the methods).

Conclusions

1. ECD works as expected, generating the specific z_n-57 fragment for the all the peptides with isoD in the sequence and differences in the y fragment intensities were observed for isoD and D peptides with UVPD at 193 nm.
2. At present, calibration curves need to be generated using synthetic peptides for the quantitation of isoD.
3. The equations shown in the results and discussion section for both the ECD and UVPD data are needed to minimise observed fluctuations in the calibration curve generated.
4. The calibration curves were easily obtained with good linearity (R^2>0.99), which is useful to quantify D and isoD in the digestion sample.

References

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Acknowledgements

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