Detecting isoAspartyl Residues in Proteins by Electron Capture Dissociation

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A post-translational modification (PTM) of proteins frequently overlooked is the non-enzymatic deamidation of asparaginyl residues that results in a mixture of aspartyl (Asp) and isoaspartyl (isoAsp) residues in place of the asparaginyl residue.¹ Deamidation is believed to be an aging pathway for proteins leading to their inactivation, misfolding and aggregation.² Formation of the isoAsp residue is believed more damaging than the change of functionality associated with Asp formation because of the insertion of the methylene group into the protein backbone. Methods to detect isoAsp residues thus far include Edman degradation, HPLC (high-performance liquid chromatography), and immunological methods which all tend to be lengthy and insensitive. Using mass spectrometry would be beneficial because of its high sensitivity however the two forms of aspartic acid are isomeric. Mass spectrometry (MS) methods developed using collisionally activated dissociation (CAD) rely on the relative abundances of b and y cleavages adjacent to the Asp/isoAsp residues and therefore require control peptides for differentiation of the two forms.³ Recently, Fourier transform mass spectrometry (FTMS) with electron capture dissociation⁴ (ECD) has shown the ability to differentiate isoAsp from Asp residues in synthetic peptides using diagnostic ions unique to each form; the cₙ₊58 and zₙ₋₅₇ ions indicate the presence and position of isoAsp residues and the (M+nH)(n-1)+•-60 (neutral loss of the Asp side chain from the reduced precursor) indicates the presence of Asp residues (n is the N-terminal amino acid neighbor to isoAsp/Asp and l is the length of the peptide and numbers refer to Daltons).⁵ Showing the fragmentation patterns in peptides from proteins will demonstrate the applicability of this method for differentiating the two forms of aspartic acid in whole proteins without the need for control peptides.

An ESI-qQq-FTMS using a 7T magnet equipped with a dispenser cathode electrode was used to analyze peptides by ECD. The peptides were isolated then irradiated with low energy (~0.2eV) electrons for 0.1-0.3 seconds to generate ECD fragments followed by detected using a typical ESI-FTMS pulse sequence. The tryptic digest of cytochrome C was incubated for 14 days at pH 11 (20mM CAPS titrated with NH₄OH) to produce the deamidated peptide. A Beckman Coulter HPLC with Vydak C18 column (250x4.6mm) was used to separate the tryptic peptides of cytochrome C using a gradient of 15-19% B in 60 minutes (A:95% ACN w/ 0.5% TFA, B: 80% ACN w/ 0.35% TFA). Calmodulin was deamidated at pH 7.4 (Tris-HCl) and 37°C for 21 days then digested with trypsin to obtain the deamidated peptide.

The deamidated tryptic peptide of cytochrome C, $^{29}$TGPNLHGLFGR$^{38}$ (m/z=585.307, 2⁺), was isolated by the front-end scanning quadrupole and subjected to ECD. The ECD spectra confirms that the peptide is a mixture of isoAsp and Asp residues at position 31 (figure 1); the z₉₋₅₇ ion (m/z=841.468, 1⁺), indicating the presence and the position of the isoAsp residue, and loss of the aspartic acid side chain from the reduced precursor ion (m/z=1110.593, 1⁺), showing the presence of an Asp residue, were present in the mass spectrum. The two forms of the peptide separated by HPLC were distinguished by ECD using the diagnostic ions indicating that deamidation resulted in 75% in favor of the isoAsp form.

Figure 1. ECD spectrum of deamidated peptide $^{29}$TGPNLHGLFGR$^{38}$ from cytochrome C. † is loss of NH₃ and CO₂ from (M+2H)⁺.
The diagnostic ions were also detected in a deamidated protein, calmodulin. Extended storage of calmodulin under physiological conditions resulted in the deamidation of an asparagine residue (N97) that is in one of the active sites of the protein and therefore available to the solvent. The multiply charged tryptic peptide of the protein containing the deamidated residue was isolated by SWIFT and subjected to ECD. ECD of the deamidated peptide $^{91}$VFKDGGNGYISAAELR$^{106}$ (figure 2) showed both the diagnostic ions indicating the presence and position of the isoAsp residue ($c_{6}^{+}+58$ and $z_{10}^{10-}57$). The diagnostic ion for the Asp residue was very intense due to the additional Asp residues in the peptide and therefore less informative about the presence of aspartic acid at position 97.

ECD of deamidated peptides from proteins and peptides from deamidated proteins show the isoAsp and Asp residue fragmentation pattern therefore showing the applicability of the method to the analysis of whole proteins.

References:


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