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Publication date: 2014

Document Version
Publisher’s PDF, also known as Version of record

Link to publication from Aalborg University

Citation for published version (APA):

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Optimizing the Identification of Citrullinated Peptides by Mass Spectrometry

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Introduction

Citrullination is a PAD-enzyme catalyzed deamination of arginine, yielding the non-standard amino acid citrulline.1

Arginine

\[
\text{HN} \quad \text{NH} \quad \text{NH}
\]

\[
\text{H} \quad \text{N} \quad \text{H}
\]

\[
\text{N}
\]

\[
\text{H}
\]

\[
\text{R}
\]

\[
\text{R}
\]

PAD Citrullination

\[
\text{H}_2\text{O}, \text{Ca}^{2+}
\]

\[
\text{O} \quad \text{NH} \quad \text{NH}
\]

\[
\text{O} \quad \text{H} \quad \text{N}
\]

\[
\text{N}
\]

\[
\text{H}
\]

\[
\text{R}
\]

\[
\text{R}
\]

Citrulline

Protein citrullination has been associated with several diseases and auto-antibodies against citrullinated proteins are today used as an important clinical biomarker in rheumatoid arthritis.2,7 The site-specific characterization of citrullination using mass spectrometry remains problematic, especially as citrullination and deamidation of asparagine or glutamine results in the same mass increase of +0.984016 Da. The verification, therefore, often relies on a trypsin miscleavage after citrulline.2 Furthermore, the mass increase is close to that of a neutron, +1.08665 Da.

However, tryptic cleavage after citrulline has in some cases been reported, so we here investigate the cleavage properties of trypsin after a citrulline residue.

Method

24 synthetic peptide sets containing either arginine or citrulline were analyzed (JPT Peptide Technologies GmbH). The peptide sequences originated from disease-associated in vivo citrullinated proteins. In-solution tryptic digestion was performed with sequencing grade trypsin (Promega). 1 pmol sample was analysed using ESI LC-MS/MS in positive ion mode, on a hybrid microQTOF mass spectrometer (Bruker). The peptides were separated using an in-house packed 10 cm reversed phase C18 column (Dr. Maisch; repnost®-pur C18-AQ) with acetonitrile.

Extracted ion chromatograms (XIC) were constructed in Bruker Daltonics DataAnalysis v 3.4, with all predicted tryptic peptides ±/ m/z 0.01, under the assumption that trypsin cleaves after arginine, lysine and citrulline.

Results and Discussion

In situ digestion results were compared to the empirical data. For SP 10, prior to digestion only the synthetic peptide is detected and after digestion, peptides corresponding to PAPDR, LLLASPR and SCYK are detected, corresponding to a successful complete cleavage after 495Arg. This is not the case after digestion of SP 10Cit, where peptides corresponding to PAPDR and LLLASPCitSCYK are detected. All investigated peptides demonstrate this behavior.

Our results clearly demonstrate the inability of trypsin to cleave after citrulline residues. Hence, a miscleavage indicates the presence of the PTM. Furthermore, the shift in retention time between the citrulline and arginine peptides was large enough for 22 of the 24 peptides to ensure that both peptides could be identified.

References

4. D. Derfyn, et al., “Citrullination increases antigenicity of citrullinated proteins and decreases their biological activity” a,b, m/e pair 53, 0.005 (ESI/MS/MS).
5. Derfyn D, et al., “Citrullination increases antigenicity of citrullinated proteins and decreases their biological activity” a,b, m/e pair 53, 0.005 (ESI/MS/MS).