Impaired Tryptic Proteolytic Activity at Citrullinated Amino Acids

Bennike, Tue; Lauridsen, Kasper; Andersen, Vibeke; Birkelund, Svend; Stensballe, Allan

Publication date:
2013

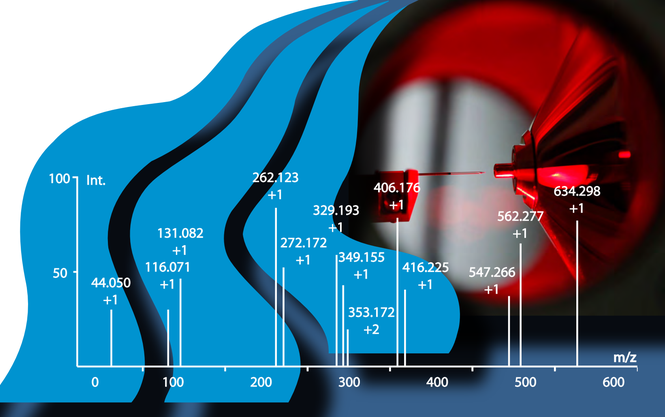
Document Version
Publisher's PDF, also known as Version of record

Impaired Tryptic Proteolytic Activity at Citrullinated Amino Acids

Tue Bennike[1], Kasper B. Lauridsen[1], Vibeke Andersen[2], Svend Birkelund[1], Allan Stensballe[1]

[1]Department of Health Science and Technology, Aalborg University, Denmark
[2]University of Southern Denmark

AALBORG UNIVERSITY
AALBORG · ESBJERG · COPENHAGEN

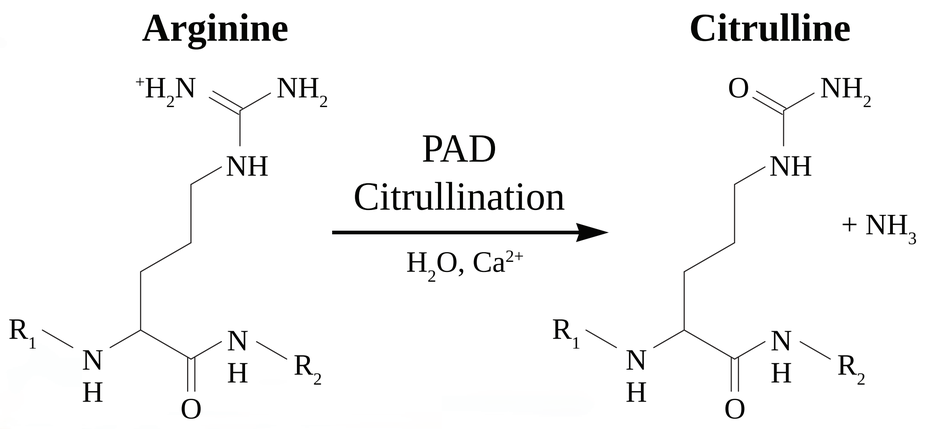


AIM

- Investigate the cleavage properties of trypsin after a citrulline residue.
- Investigate the behavior of citrullinated peptides by reversed phase chromatography.
- Propose a verification strategy for detected citrullinated peptides in a MS workflow.

Introduction

Citrullination is a PAD-enzyme catalyzed deimination of arginine, yielding the non-standard amino acid citrulline along with ammonia.[1]



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] 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 tryptic miscleavage after citrulline.[3] Furthermore, the mass increase is close to that of a neutron, +1.08665 Da, making it challenging to detect both unmodified and modified peptides when coeluting, due to the typical isolation window used (m/z 1-3).

However, tryptic cleavage after citrulline has in some cases been reported, so we here investigate the cleavage properties of trypsin after a citrulline residue and the behavior of citrullinated peptides by reversed phase chromatography.

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, some reported as being C-terminal tryptic citrullinated peptides. In-solution tryptic digestion was performed in 5% acetonitrile, 50 mM ammonium bicarbonate and 0.5 µg crude peptide was added to 0.05 µg sequencing grade trypsin (*Promega*). The samples were digested for 12 h at 37 °C and acidified with formic acid. 1 pmol sample was analysed using ESI LC-MS/MS in positive ion mode, on a hybrid microQTOF mass spectrometer (*Bruker*). The peptides were seperated using an in-house packed 10 cm reversed phase C18 column (*Dr. Maisch; reprosil-pur C18-AQ*), and eluated with a linear gradient of 98% solvent A (0.1% formic acid, 0.005% heptafluorobutyric acid) and 2% solvent B (90% acetonitrile, 0.1% formic acid, 0.005% heptafluorobutyric acid), which was raised to 40% solvent B over 30 min, at a constant flow rate of 200 nL/min. 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.

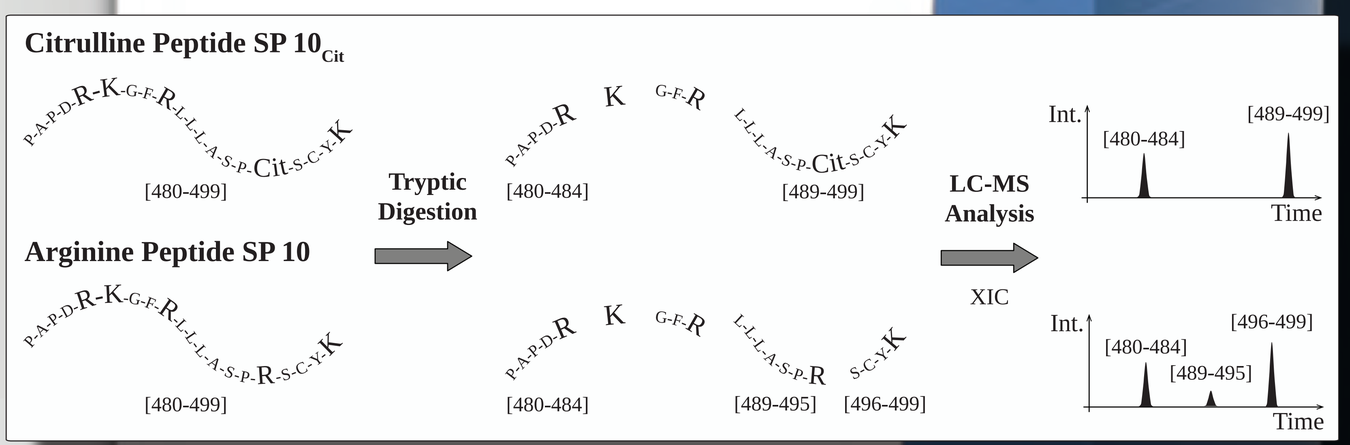
Conclusion

- Our results clearly demonstrate the inability of trypsin to cleave after citrulline residues. As a result, a miscleavage can be used to distinguish a citrullination from a deamidation of asparagine or glutamine.
- The shift in retention time was, for 22 of 24 peptides large enough to ensure that both peptides could be identified.

SP #	Sequence	Protein ID	% RT shift
1	DS **R/Cit** GNPTVEVDLFTS**K**GLF**R**	P06733.2	2.4
2	DPS **R/Cit** YISPDQLADLY**K**SFI**K**	P06733.2	3.0
3	EELGS**K**A**K**FAG **R/Cit** NF **R/Cit** NPLA**K**	P06733.2	1.6
4	VTTST**R**TYSLGSAL **R/Cit** PSTS**R**	AAH66956	1.4
5	EQL**K**GQG**K**S **R/Cit** LGDLYEEEM**R**	AAH66956	2.6
6	NM**K**EEMA**R**HL **R/Cit** EYQDLLNV**K**	AAH66956	1.4
7	NM**K**EEMA **R/Cit** HL**R**EYQDLLNV**K**	AAH66956	2.0
8	LHVA **R/Cit** SEMD**K**V **R/Cit** VFQAT **R/Cit** G**K**	NP_036519.2	3.6
9	GL**K**EFPI**K** **R/Cit** VMGPDFGYVT**R**	NP_036519	3.4
10	PAPD**R**K**G**F**R**LLLASP **R/Cit** SCY**K**	NP_036519	2.0
11	LS **R/Cit** TV**R**CTCISISNQPVNP**R**	P02778.2	1.6
12	EMHG**K**NWS**K**LC **R/Cit** DCQVIDG**R**	ACB10579	1.6
13	SGVT**K**AISSPTVS **R/Cit** LTDTT**K**	ACB10579	1.4
14	AEGGGV **R/Cit** GP**R**VVE **R/Cit** HQSAC**K**	P02671	0
15	SHHPGIAEFPS **R/Cit** GK**S**SSYS**K**	P02671	0.4
16	FTSSTSYN **R/Cit** GDSTFES**K**SY**K**	P02671	1.0
17	A **R/Cit** HGFLP **R/Cit** H**R**DTGILDSIG**R**	P02686.3	2.6
18	LS**R**FSWGAEGQ **R/Cit** PGFGYGG**R**	P02686.3	2.0
19	PGFGYGG **R/Cit** ASDY**K**SAH**K**GF**K**	P02686.3	0.6
20	LS**K**IF**K**LGG **R/Cit** DS**R**SGSPMA**R**	P02686.3	0.6
21	Y **R/Cit** VYCDMNTENGGWTVIQN**R**	P02675	0
22	MYLIQPDSSV**K**PY **R/Cit** VYCDM**R**	P02675	3.4
23	EAPSL **R/Cit** PAPPPISGGGY**R**AR	P02675	2.0
24	SI **R/Cit** YLQEIYNSNNQ**K**IVNL**K**	P02679.3	3.0

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 495_{Arg}. This is not the case after digestion of $SP\ 10_{Cit}$, 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 can be used together with the mass shift, retention time shift to validate the PTM. We are currently investigating if a 43 Da neutral loss by citrulline[5] can be used in a method to trigger precursor selection on a Q Exactive. Furthermore, the shift in retention time between the citrulline and arginine peptides was large enough for 22 of the 24 peptides to ensure that coelusion is not occurring to a detectable extend, ensuring that both peptides can be identified.

References

[1] *W. R. Fearon, "The carbamido diacetyl reaction: a test for citrulline.", Biochem J. 33, 902–907 (1939).*
[2] *G. A. Schellekens, et. al., "Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies", J. Clin. Invest. 101, 273 (1998).*
[3] *M. De Ceuleneer, et. al., "In vivo relevance of citrullinated proteins and the challenges in their detection", PROTEOMICS 12, 752–760 (2012).*
[4] *P. K. Gregersen, et. al,, "Circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis", Arthritis Res Ther. 10, 94 (2008).*
[5] *G. Hao, et. al., "Neutral loss of isocyanic acid in peptide CID spectra: a novel diagnostic marker for mass spectrometric identification of protein citrullination". J. Am. Soc. Mass 20, 4, 723–727 (2009).*
[6] *(submitted) T. Bennike, et. al., "Impaired Tryptic Proteolytic Activity at Citrullinated Amino Acids", Eur. J. Mass (2013)*