The citrulline effect in the dissociation of deiminated peptides

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Introduction

Protein citrullination is a post-translational modification (PTM) converting certain arginines (Arg, R) to **citrullines (Cit, X).**[1] This results in a +0.9840 Da mass increment and the loss of a positive charge per conversion. Citrullination plays roles in gene and immune regulation, skin homeostasis, the tight packaging of axons and fertility. Overcitrullination is found to be associated with several diseases.

The only method that is currently capable of determining the position of citrullinated residues with acceptable sensitivity and selectivity is MS/MS based proteomics.[2] However, low concentration of the modified species and isobaric interference from deamidation of Asn and Gln residues often hinder the identification. So far, there are only two studies in the literature aiming to explore the fragmentation characteristics of citrulline-containing peptides, one of them on the peptide level,[3] the other from a proteomic approach.[4]

We previously coined the term **citrulline effect** and emphasized its usefulness in the confirmation of modification sites.[3] Citrulline effect is an amino acid effect resulting in a **preferred cleavage of the Cit-Aaa bond at low collision energies**. If the Cit was located at the *C*-termini, an Aaa-Cit cleavage would be observed instead.

In this work, we aim to examine and illustrate the citrulline effect on model peptides and on peptides originated from a proteomic data set published recently by Lee et al.[4]

Methods

The peptide series used for our experiments are summed up in **Table 1**. The first set was used for ionization efficiency and charge state distribution studies, the second peptide set was selected from epitope regions of proteins associated with *rheumatoid arthritis*.[3] These two sets were synthetic peptides. Mass spectrometric measuring conditions are summarized in **Table 2**.

The third set of peptides were originated from a database of ~1000 citrullinated tryptic peptides found in various human tissues. The database was recently created and published by Lee et al. PRIDE converter and PRIDE inspector were used for data-processing and visualization, respectively. All spectra were acquired at high resolution and with high mass accuracy except for the ones performed in a quadrupole ion trap.

Table 1. Peptide series used for the examination of Cit effect

Set 1	Set 2	Set 3
AAAA, AXAA GGGG, GXGG	11 clinically relevant peptides	~1000 peptides from proteomic samples

Table 2. Conditions of MS analysis of peptide samples sets 1 and 2

Solvent	CH_3CN-H_2O (1:1 *v*/*v*), 0.1 % HCOOH
Injection	direct
Flow rate	10 µL/min
Source	ES+
Fragmentation	low energy CID
Instrument type	QIT, QqTOF, Q-Orbitrap

Results and discussion

In this presentation we demonstrate the presence and importance of citrulline effect in the MS/MS analysis of citrullinated (deiminated) peptides. Our key results concerning the citrulline effect can be summarized in the following points:

- **Cit-Aaa bonds are preferably cleaved at low-energy CID (Figure 1-3)**
- **The corresponding *y* ion and not the *b* ion is generated in almost all tryptic peptides**
- **>10 % of tryptic Cit-peptides displayed the corresponding *y* ion from Cit effect as the *largest* sequential peak**
- **Cit effect is similar in intensity to Pro effect**
- **Cit effect is the most significant fragmentation pathway if:**
  - Aaa = Pro, His (additional Pro and His effect)
  - there is no Pro in the sequence and Aaa = Gly > Ser ≃ Asp > Ala > Glu

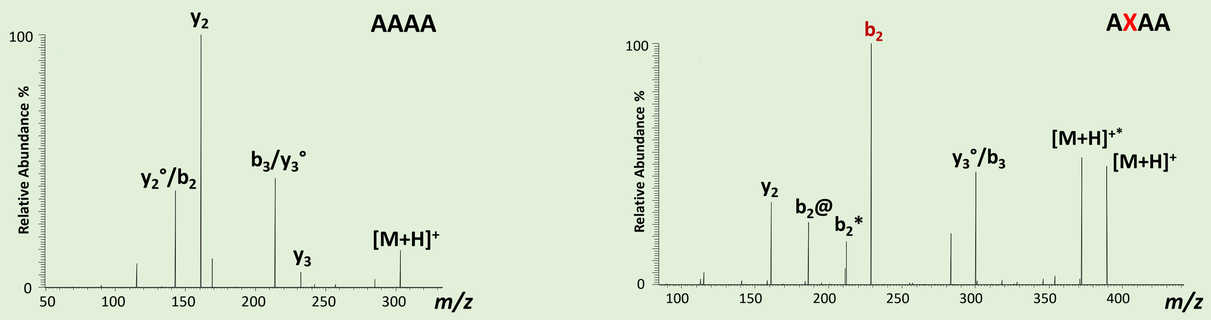

Figure 1. The tandem mass spectra of peptides AAAA and AXAA measured on a Thermo Scientific Q Exactive Focus orbitrap instrument at a normalized collision energy of 15 V. ***Different base peaks imply an altered fragmentation pathway for the citrulline-containing peptide***. X stands for citrulline, @ * and ° denote a loss of HNCO, NH_3 or H_2O respectively.

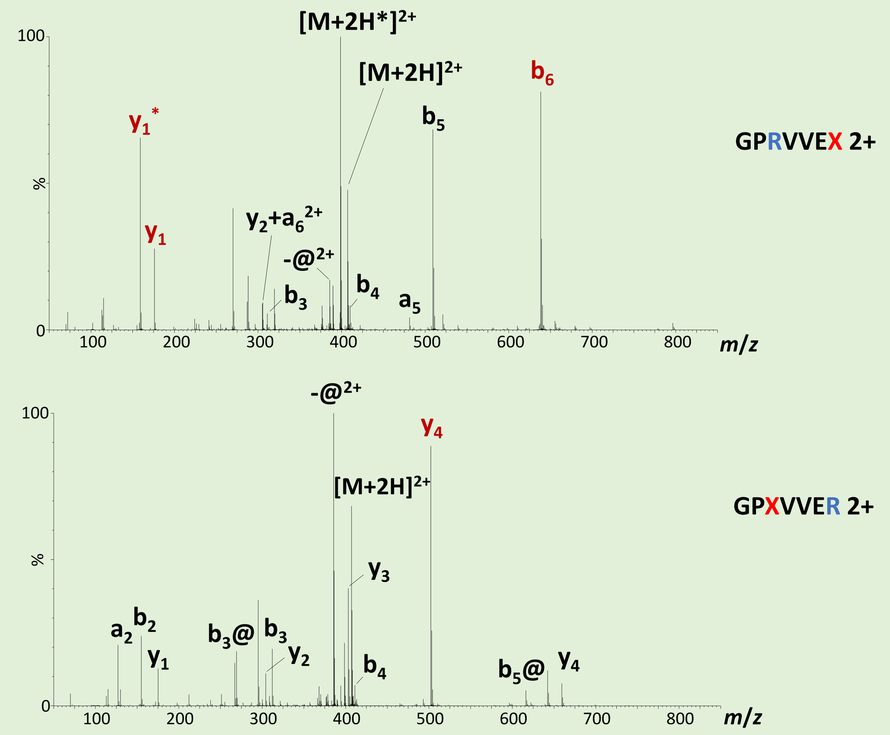

Figure 2. The tandem mass spectra of doubly charged peptides GPRVVEX and GPXVVER measured on a Waters QTOF Premier instrument at 10.0 eV collision energy[3]. ***The peaks originating from Cit-Aaa cleavage are labeled with red color.*** X stands for citrulline, @ and * denote a loss of HNCO and NH_3 respectively.

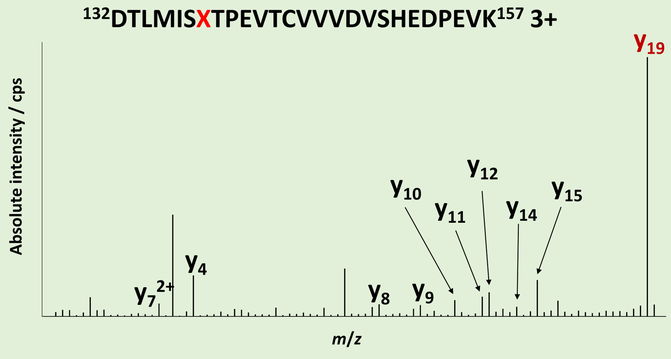

Figure 3. The tandem mass spectrum of a triply charged, iodoacetamide derivatized peptide of the tryptic digest of immunoglobulin heavy constant gamma 1 (IGHG1)[4]. ***The base peak is the y_{19} ion originated from the Cit-Aaa cleavage.***

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