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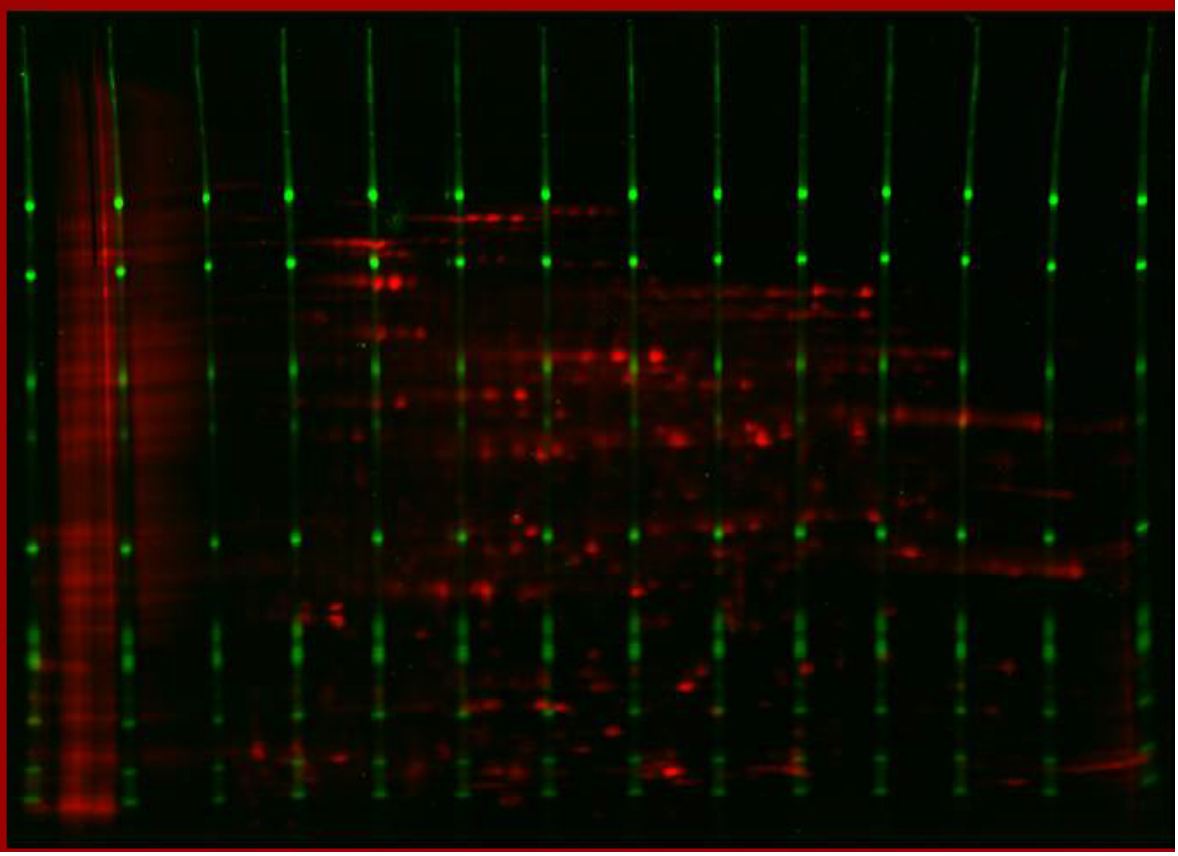

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Germany
https://campus.uni-muenster.de/cu-proteomics
Edited by Simone König
CUP/IZKF, Röntgenstr. 21, 48149 Münster
koenigs@uni-muenster.de
Ph. +49-251-8357164

Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Protocol

Target analysis for the Cys-cluster tryptic peptide of acylated transglutaminase 1

Simone König[1], Malte Bayer[1] and Henning Wiegmann[2]*

[1] Core Unit Proteomics, Interdisciplinary Center for Clinical Research,

[2] Department of Dermatology, University Hospital Münster, Münster, Germany

*Corresponding author: koenigs@uni-muenster.de

Abstract

Transglutaminase 1 (TGase1) is modified by myristoylation and palmitoylation supposedly in its cysteine cluster region. In order to monitor acylation in protein expression we developed a target mass spectrometry method using synthetic peptide SFWAR$C_{Palm}C_{am}GC_{am}C_{am}SC_{am}$R as a standard. It is palmitoylated at Cys6 and carbamidomethylated on the remaining cysteines to enable its use as surrogate peptide for the tryptically generated peptide from TGase1. The method allows the specific detection of the peptide based on its retention time in reversed-phase chromatography and its gas phase fragmentation pattern. In addition, the presence of a myristoyl group and of the peptide with no missed cleavages was programmed.

Introduction

Transglutaminase 1 (TGase1) plays a decisive role in the assembly of the cornified cell envelope in terminally differentiating epidermal keratinocytes in the transition between the two upper layers of the epidermis, the stratum granulosum and stratum corneum [1]. In human, the absence of TGase 1, due to loss-of-function mutations within the *TGM1* gene encoding TGase 1, results in the disorder referred to as TGase 1-deficient autosomal recessive congenital ichthyosis (ARCI type 1) that presents itself immediately after birth. The disorder is characterized by an initial redness of the skin that develops into large brown, plate-like thick horny skin covering the entire body or at least large areas [2]. It has been demonstrated that the enzyme is modified by myristoylation and palmitoylation supposedly in its cysteine cluster region using autoradiography, hydrolysis, chromatography and MALDI-TOF mass spectrometry (MS, Fig. 1) [3]
In order to monitor acylation in protein expression we set up a target MS method using synthetic peptide (SP) SFWAR$C_{Palm}C_{am}GC_{am}C_{am}SC_{am}$R as a standard. It is palmitoylated at Cys6 and carbamidomethylated on the remaining cysteines to enable its use as surrogate peptide for the tryptically generated peptide from TGase1. It was synthesized with one missed cleavage, because trypsin activity may be limited next to the palmitoylated cysteine residue.

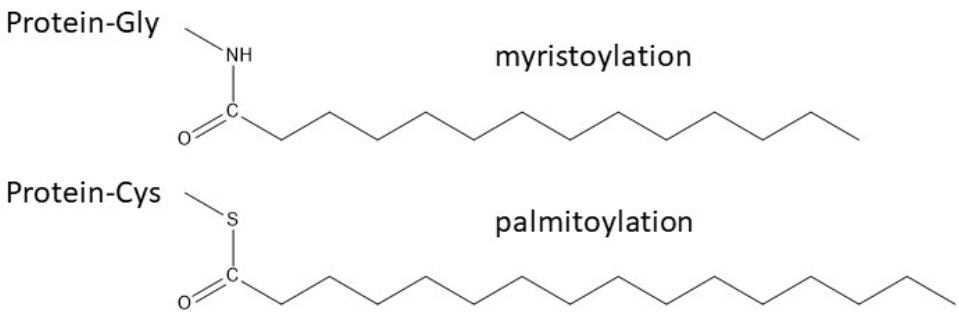

Figure 1: Schematics for the palmitoylation on Cys and the myristoylation on the N-terminal Gly.

Experimental

The peptide was synthesized by Peptide Special Laboratories, Heidelberg, Germany. The synthesis was difficult and did not produce pure product at well-known concentration. Quantification with this material was thus not an option, but we were able to use it to determine the retention time in reversed-phase liquid chromatography (LC) and the SP gas phase fragmentation behavior for subsequent target analysis. To that end, the peptide was dissolved in 10% acetonitrile (ACN) containing 0.1% formic acid (FA). Experiments were performed with Synapt G2 Si ion mobility mass spectrometer with M-Class UPLC (Waters Corp.; 30 min gradient from 3 to 40% organic, solvent system 100% water versus 100% ACN, both containing 0.1% FA; flow rate 300 nl/min; trap column V/M Symmetry C18 100 Å 5 µm, 180 µm x 20 mm; reversed phase column HSS T3 1.8 µm 75 µm x 200 mm). The doubly and the triply-charged peptide ions were selected for MS/MS (*m/z* 649.96, collision energy ramp 7-16 V; *m/z* 974.43, collision energy ramp 38-48 V; 0.2 s). Further target masses for investigations of digested TGase1 included:

- *m/z* 650.77 (0 missed cleavage, 1 palmitoylation)
- *m/z* 727.36 (0 missed cleavage, 1 palmitoylation, 1 myristoylation)
- *m/z* 701.02, *m/z* 1051.02 (1 missed cleavage, 1 palmitoylation, 1 myristoylation)

For upconcentration of acylated peptides, a solid-phase extraction method was developed. Tryptic peptides in 50% ACN containing 0.1% FA were loaded onto a ZipTipC18. Following washing of the solid phase 10 times with 20% ACN, 0.1% FA, the bound material was eluted with 100% ACN. The solvent was evaporated in vacuo using a speedvac and the sample was redissolved in 10% ACN containing 0.1% FA prior to analysis.

Results

The LC gradient applied is routinely used to measure pools of tryptic peptides. Under those conditions, the hydrophobic SP eluted considerably later (42.5 min, Fig. 2) than most regular peptides, which is not surprising considering its hydrophobicity. It was also observed that the peptide was not well resolved in nanoflow LC and, moreover, difficult to remove from the column requiring lengthy washing procedures.

The overview scan shows a dominant triply-charged parent ion and the easy loss of three water molecules likely from the C-terminus and the two serine residues. The triply-charged ion is best suited for MS/MS analysis due to its abundance (Fig. 3). Its spectrum is characterized mainly by the immonium ion for tryptophan and the doubly-charged $y"_{11}$ fragment ion with and without the palmitoyl residue. Obviously, fragmentation occurs preferentially at the bulky Trp residue and the lipid is then removed easily. These ions can be well observed even at low peptide concentrations and serve as indicators for the presence of the peptide.

Due to the difficulties in peptide synthesis it was not feasible to generate all possible peptides relevant for the investigation of TGase1, namely the shorter peptide with no missed cleavage and the peptide containing additional myristoylation as proposed [3]. However, using the knowledge of the retention time of SP, it can be assumed that both doubly acylated SP and SP with no missed cleavage should elute in about the same time frame due to the hydrophobicity of the lipid component; the former a little later than SP, the latter somewhat earlier. For all possibilities mass target functions were programmed.

Conclusion

MS-based target analysis for the Cys-cluster region of TGase1 using a derived synthetic tryptic peptide was developed. The method serves as quality control experiment for the presence of this peptide in varying preparations of TGase1 after tryptic digestion based on its retention time and MS/MS fragmentation pattern. The protocol was validated for the SP and extrapolated for the presence of both a higher acylated and a shorter form.

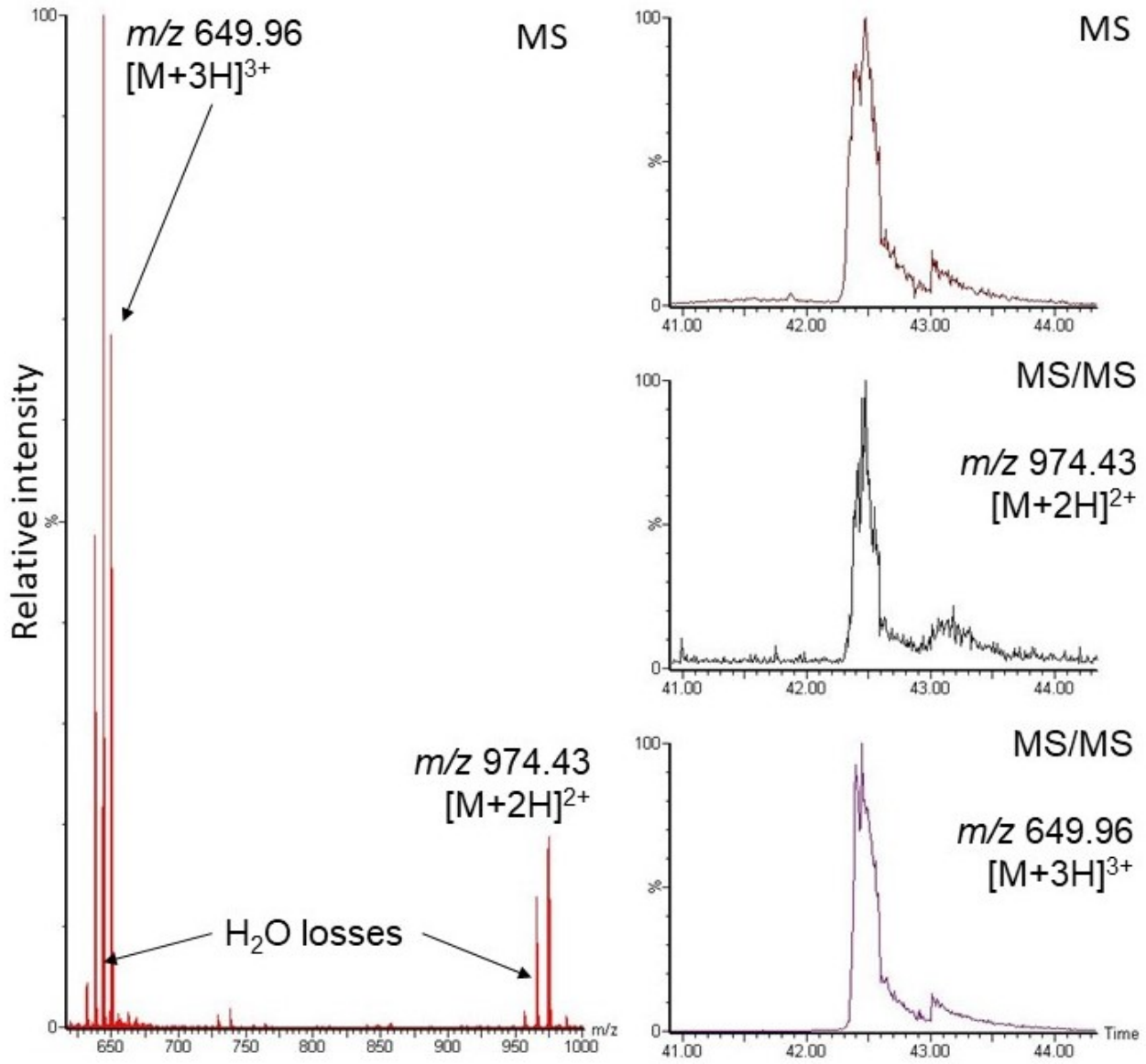

Figure 2: Chromatogram with traces for doubly and triply-charged parent ion and MS scan and MS spectrum for SP.

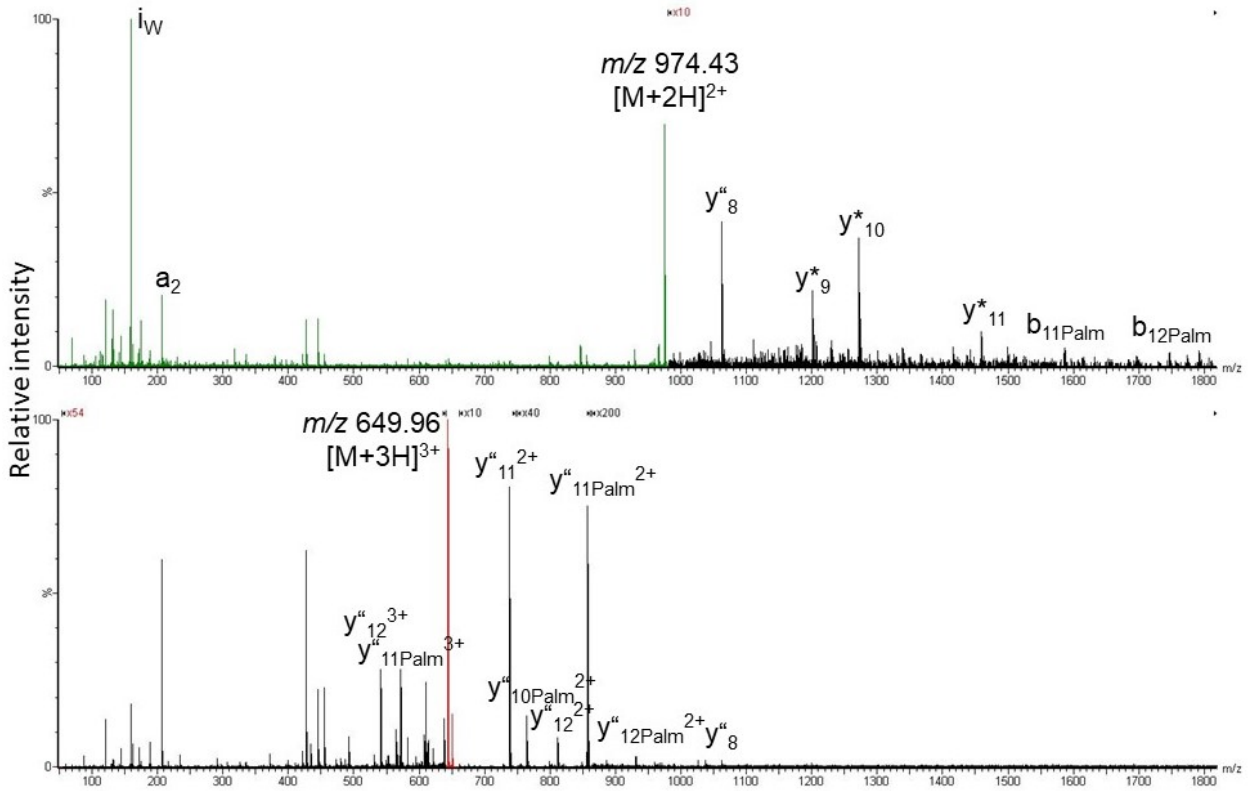

Figure 3: MS/MS spectra of doubly and triply-charged SP ions. Note zoom areas.

References

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