Simone Nicolardi[1], David P.A. Kilgour[2], Natasja Dolezal[3], Jan W. Drijfhout[3], Manfred Wuhrer[3], Yuri E.M. van der Burgt[1]

[1] Center for Proteomics and Metabolomics, [3] Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands

[2] Department of Chemistry, Nottingham Trent University, United Kingdom

Disentangling distorted distributions - improving z' ion assignment confidence and structural characterization of proteins by top-down MALDI–in-source decay MS

Introduction

Protein structural analysis by mass spectrometry relies on the use of different fragmentation techniques to provide comprehensive determination of protein sequence, including any post-translational modifications. MALDI, combined with in-source decay (ISD) fragmentation (Figure 1 and 2) and FT-ICR-MS, enables top-down C- and N-terminal sequencing of proteins. Here, we evaluate why isotopic distributions of z' ions, in top-down protein MALDI-ISD-FT-ICR mass spectra, deviate from theoretical profiles: a result of interference from the overlap with isotopic distributions of both *z* and *y*-NH$_3$ ions (which can co-occur and are isomerous with each other). Recognizing this interference can result in higher confidence structural characterization of proteins, which is important for manufacturing of pharmaceutical products such as therapeutic monoclonal antibodies (mAbs).

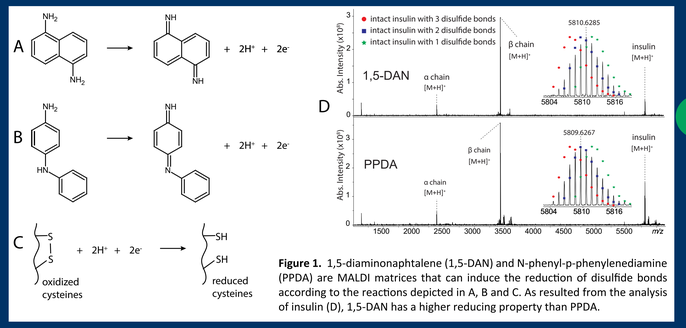


Figure 1. 1,5-diaminonaphtalene (1,5-DAN) and N-phenyl-p-phenylenediamine (PPDA) are MALDI matrices that can induce the reduction of disulfide bonds according to the reactions depicted in A, B and C. As resulted from the analysis of insulin (D), 1,5-DAN has a higher reducing property than PPDA.

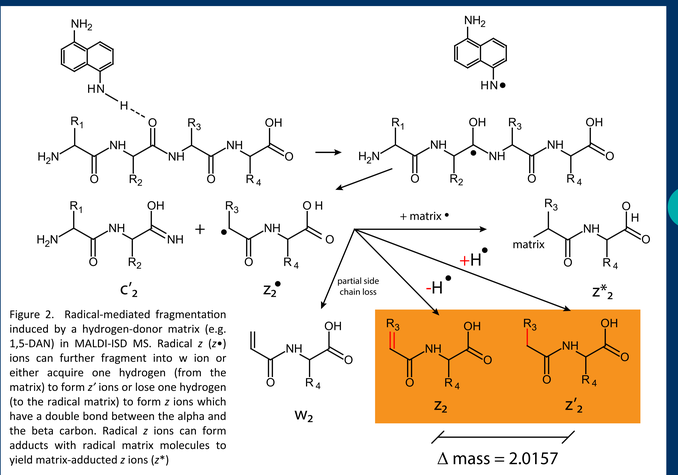
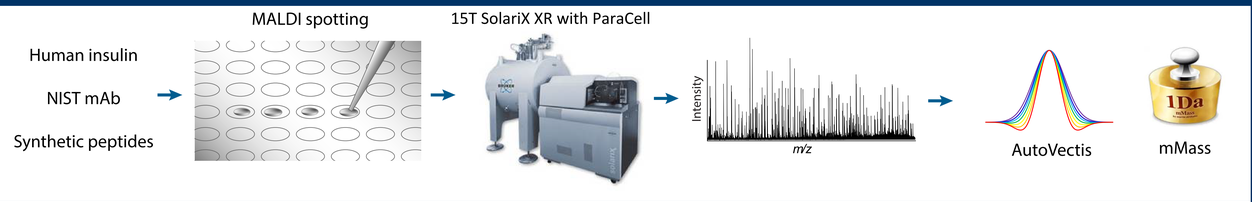


Figure 2. Radical-mediated fragmentation induced by a hydrogen-donor matrix (e.g. 1,5-DAN) in MALDI-ISD MS. Radical *z* (*z*•) ions can further fragment into w ion or either acquire one hydrogen (from the matrix) to form *z'* ions or lose one hydrogen (to the radical matrix) to form *z* ions which have a double bond between the alpha and the beta carbon. Radical *z* ions can form adducts with radical matrix molecules to yield matrix-adducted *z* ions (*z**)

Method

MALDI-(ISD) MS measurements were performed on a 15T solariX XR FT-ICR MS system (Bruker Daltonics). 1,5-DAN and PPDA were used as MALDI matrices. NIST mAb was used as model compound. MALDI-ISD MS mass spectra of NIST mAb were processed in both magnitude (mFT) and absorption (aFT) modes, using AutoVectis (Spectroswiss). A figure-of-merit (FoM) was calculated to provide a measure of how closely an isotopic peak distribution matched the theoretical distribution. Two synthetic peptides that contain either normal or deuterated alanine residues were used to investigate the presence and unravel the identity of isomeric *z* and *y*-NH$_3$ fragment ions.



The isotopic distributions of z' ions are distorted due to a partial overlap with isomeric *z* and *y*-NH$_3$ ions

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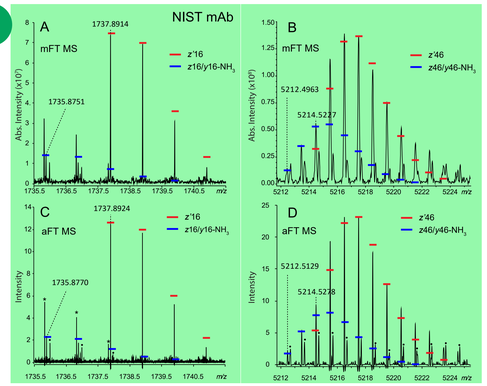

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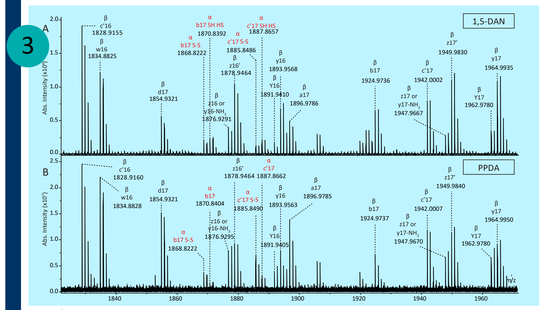

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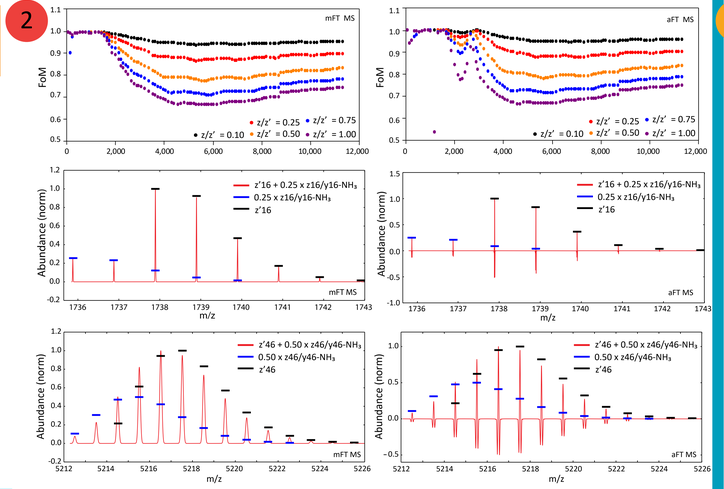

Results

(1) Isotopic distributions of z' and *z*/*y*-NH$_3$ ions detected in mFT (A and B) and aFT (C and D) MALDI-ISD FT-ICR MS spectra of the heavy chain of NIST mAb. The colored marks indicate theoretical isotopic distributions. The FoM values obtained for aFT isotopic distributions of z'16 and z'46 ions were 0.95 and 0.81, respectively.

(2) Evaluation of the apparent distortions of the isotopic distribution of z' ions induced by isomeric z and y-NH$_3$ ions on theoretical MALDI-ISD FT-ICR mass spectra of the heavy chain of NIST mAb. The goodness of the isotopic distribution of each z' ions, expressed as FoM, was calculated considering different z/y-NH$_3$-to-z' ratios. FoM values lower than 1 indicate a deviation of the isotopic distributions induced by the presence of z/y-NH$_3$ ions.

(3) Evaluation of isomeric z and y-NH$_3$ fragment ions in MALDI-ISD FT-ICR MS spectra of human insulin analyzed using A) 1,5-DAN and B) PPDA as MALDI matrices.

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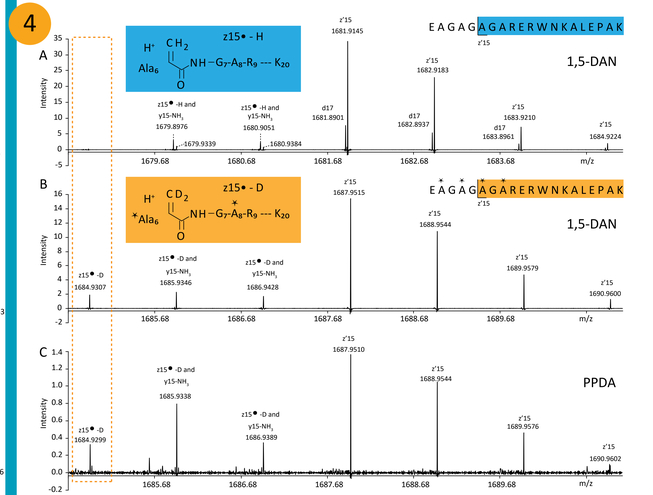

(4) Evaluation of isomeric z and y-NH$_3$ ions in MALDI-ISD FT-ICR mass spectra obtained from the analysis of a pair of peptides, A) one with normal alanine (m/z 2082.0814) analyzed with 1,5-DAN and one with heavy alanine (m/z 2094.1591) analyzed with both B) 1,5-DAN and C) PPDA. The abstraction of one deuterium instead of one hydrogen from alanine-6 led to the detection of an additional peak at m/z 1684.9307 (or m/z 1684.9299). The isotopic distribution on z15*-D resulted distorted as a consequence of the presence of y15-NH$_3$ ions.

Conclusion

In this study, we showed that the detected isotopic distribution of z' fragment ions, generated by MALDI-ISD FT-ICR MS of proteins, can be affected by the presence of interfering z and y-NH$_3$ fragment ion isotopic distributions. The z and y-NH$_3$ fragment ions are isomeric but are generated by different fragmentation paths. The evaluation of the presence of these ions increases the confidence of the identifications and explains decreased FoM values of certain z' ions.

Reference Nicolardi *et al. Anal. Chem.* 2020, 92, 8, 5871-5881