

Tandem mass tags for quantitative analysis of N-glycosylation

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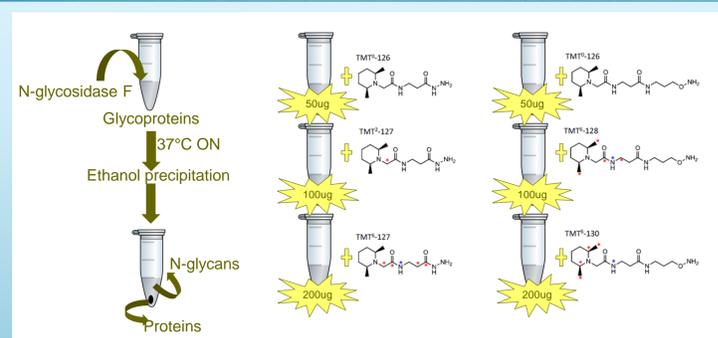
Introduction

Among the organic components, carbohydrates are the most abundant on the Earth and they can be found in nature as pure components or as glycoconjugates. 50% of mammalian proteins are glycosylated. Oligosaccharides post-translationally conjugated to proteins are characterized by a high microheterogeneity, which increases variation among gene products, with a total of 13 monosaccharides and 8 aminoacids forming at least 41 types of different glycosidic linkages [1]. Therefore glycoproteins exist as complex mixtures of glycosylated variants (glycoforms). Anomalous pattern of glycosylation may be due to physiological events or pathological conditions. For this reason it is very important to develop qualitative and quantitative strategies for the analysis of glycosylation. However glycans analysis is not an easy task, because they do not contain chromophores and are very hydrophilic, so a common practice is to derivatize them to increase UV absorption, fluorescence or mass spectrometric ionization. Hydrazine derivatives are generally used to derivatize carbohydrates, leading to hydrazone derivatives that, if needed, can be reduced to open ring structures [2]. Derivatized sugars can be analysed by mass spectrometry techniques in order to produce structural information. Both ESI-MS and MALDI-MS are generally used for oligosaccharides analysis. Derivatization strongly affects ionization efficiency by introducing additional charges and giving the sugar a more hydrophobic character. Moreover, labels influence glycans fragmentation behavior and help the assignment of fragmentation spectra by tagging the reducing end [3]. Here we suggest a derivatization procedure of glycans reducing ends by novel TMT-carbonyl reactive tandem mass tags.

Strategy

N-glycans were enzymatically released from different amounts of standard chicken ovalbumin and separated from proteins by a simple ethanol precipitation. TMT reagents were added to N-glycans dry samples to a concentration of 10 mM in 80% MeOH, 20% AcOH. The reaction was carried out for 4 h at 75°C and samples were dried down. TMT-hydrazide labelled glycans were subjected to reduction in 50 mM sodium cyanoborohydride for 2.5 h at 4°C. All the samples were desalted on Hypercarb SPE Columns.

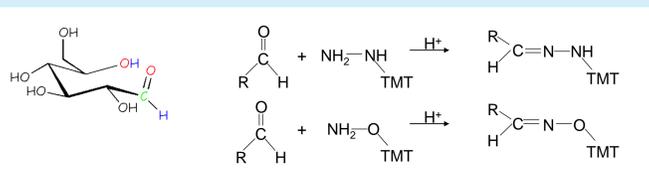
TMT-glycans were dissolved in water and mass spectra were acquired in positive ion mode using DHB as matrix on 4800 MALDI-TOF/TOF (AB Sciex) and MALDI-LTQ Orbitrap (ThermoFisher Scientific).



Results

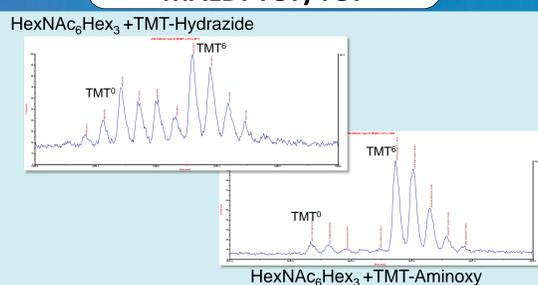
Mass spectrometry quantitative approaches already set up in proteomics can be applied to glycomics by the use of glyco-TMT reagents. These molecules, functionalized with either hydrazide or aminoxy groups, can label the reducing end of the sugars leading to the formation of hydrazones or oximes.

Light (TMT⁰) and heavy (TMT²-TMT⁶) forms are available for both hydrazide and aminoxy-functionalized reagents, as well as isobaric isotope coded reagents. For this reason it is possible to perform either quantification in full scan spectra using heavy/light comparison or quantification in tandem mass spectra using isobaric quantification.



The novel reagents aminoxy TMTs are superior to hydrazide TMTs in terms of reaction efficiency, stability of the products and accessible dynamic range of the the quantification [4].

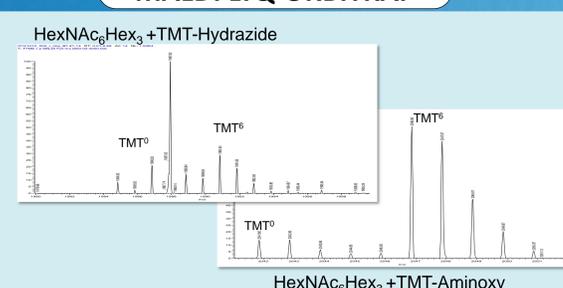
MALDI TOF/TOF



The light TMT⁰ and heavy TMT⁶ species show a mass difference of 5.0105 Da that can be used to perform quantification at the MS level. MALDI-LTQ Orbitrap revealed a better S/N ratio compared to MALDI-TOF/TOF.

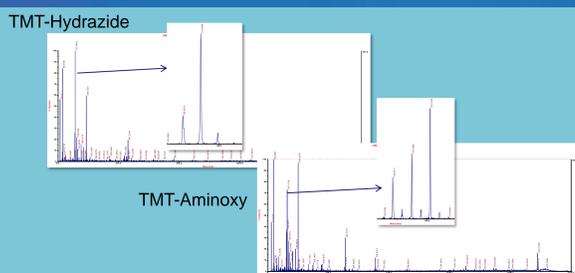
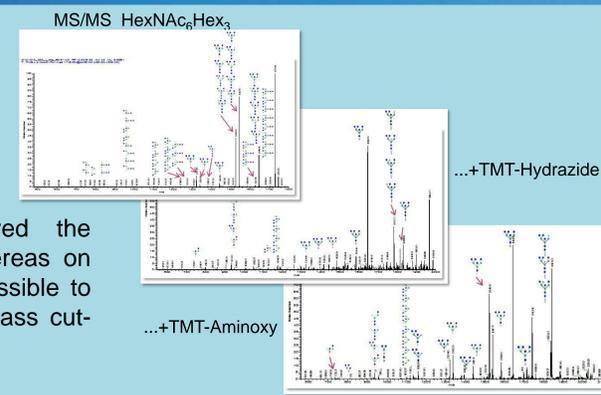
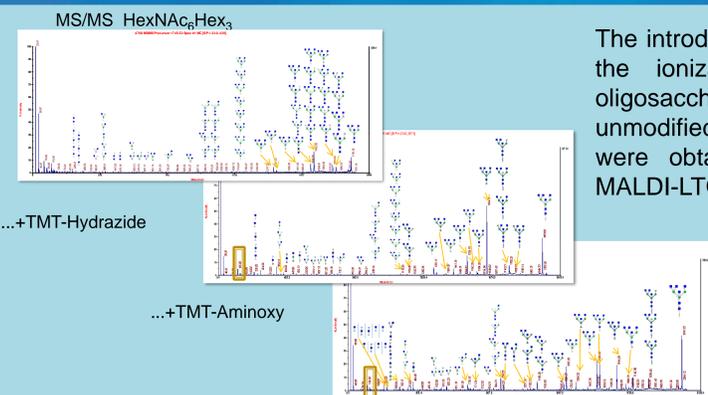
Hydrazone reduction rendered the products more stable but sometimes the reaction was not complete and the TMT-hydrazide species showed more complex profiles due to the presence of unreduced TMT-sugars.

MALDI LTQ-ORBITRAP



The introduction of the TMT label increased the ionization efficiency of the TMT-oligosaccharides compared to the unmodified ones. Better MS/MS spectra were obtained on MALDI-TOF/TOF and MALDI-LTQ Orbitrap.

MALDI-TOF/TOF analyses allowed the detection of the reporter ions whereas on MALDI-LTQ Orbitrap it was not possible to detect them because of the low mass cut-off.



Isobarically labelled glycans revealed different reporter ions, allowing oligosaccharides quantification in MS/MS on the base of the ratio between the different reporter ions. This was carried out on MALDI-TOF/TOF. Higher order MSⁿ experiments on MALDI-LTQ Orbitrap are still under investigation and may improve quantitative accuracy.



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