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 amino acids 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 glycan 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. Hydrazide 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 Scienx) 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 aminopy group, can label the reducing end of the sugars leading to the formation of hydrazone or oximes. Light (TMT 6) and heavy (TMT 7) forms are available for both hydrazone and aminopy-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 aminopy TMTs are superior to hydrazone TMTs in terms of reaction efficiency, stability of the products and accessible dynamic range of the quantification [4].

REFERENCES