KIT QUANTA - standardization kit for absolute protein quantitation: monitoring of methionine oxidation induced by chromatography separation

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Introduction

In the context of biomarker discovery and their absolute quantification in complex samples, a standardization strategy aiming to control the entire sample preparation process before LC-MS analysis would be extremely valuable. Our approach involves the use of a chimeric protein and different levels of its heavy peptides spiked at opportune moment in the sample during sample processing. Among these peptides, some containing a methionine are inserted. Methionine oxidation is a well-known protein modification occurring *in vivo* but also *in vitro* and may result in structural and functional protein alteration. Controlling this artefactual modification is then of great interest and will be possible thanks to our strategy.

Methods

To address the origin of methionine oxidation, a comparison between peptides injection by direct infusion in the mass spectrometer and by LC-MS using new columns (unused) or old columns (used) was conducted. In the latter case, an Acquity UPLC M-Class system with a nanoEase MZ symmetry C18 trap column and a NanoEase MZ HSS T3 analytical column was used. Subsequently, complex matrices (i.e. commercial HeLa protein digest) were injected on the LC system in order to see if the oxidation effect is also present when a complex matrix is used and could potentially protect the peptides from the spontaneous oxidation in the columns.

Preliminary Data

The approach was to determine where the artefactual oxidation of methionine came from. For this purpose, we sought differential oxidation levels comparing direct infusion, LC-MS using new columns and LC-MS using old ones. The direct infusion of pure synthetic peptides in a QExactive™ Hybrid Quadrupole-Orbitrap™ showed a low percentage of oxidized peptide (less than 2%). Once injected onto the M-Class system coupled online with the QExactive, this percentage jumps to higher value (50-60% dependant of the injected peptide).

The same peptides were injected on the same M-Class system on which both trapping and analytical columns were replaced by new ones. Methionine oxidation in this case, was as low as in the direct infusion, meaning that the previous use of columns has a huge influence on the oxidation of methionine on pure synthetic peptides. This effect is certainly due to the presence of metal ions in the columns that promote methionine oxidation during chromatography separation. Furthermore, at the chromatogram level, a shape difference between the oxidized peak from new columns and old columns was observed. When using new columns, the oxidized peptide peak shape is sharp whereas on old columns its shape is broadened and extended. In both cases, the native peptide peak stays sharp, proving that this modification occurs during the chromatographic separation.

The same effect was observed on the complex sample. Indeed, the percentage of oxidized peptides varies from 1 to 8% with the new columns and increases to 20% to 80% when the same HeLa digest was injected on the old columns.

These results show that the use history of a column greatly impacts the oxidation degree of peptides and need to be taken into account when studying methionine oxidation. Future works will be dedicated to the implementation of strategies that could stabilize the oxidation during chromatography separation.

Novel Aspect

New methodology to monitor within a complex sample the redox process of methionine induced by liquid chromatography separation system.