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MS-based Proteomics turns quantitative: the great future of HPLC-ICP-MS speciation techniques for this challenge

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Mass Spectrometry has become one of the most powerful analytical tools to characterise proteins. As proteomics itself, however, new advances and results in this field have been mostly qualitative. Recent approaches to obtain quantitative proteomic information exist, but they are “relative” because they rely on comparing the signals from a given peptide from two different experiments (usually using stable isotopes for labeling). Absolute quantification (only one experiment) has been scarcely addressed in proteomics. Elemental detection by ICP-MS after HPLC separation, typically used in trace element speciation, may provide a means to modern biochemists for absolute quantitative proteomics, particularly for the study of post-translational modifications (PTMs) of proteins.

For instance, it is known that abnormal Tf isoforms, commonly referred to as carbohydrate-deficient-transferrins (CDTs), are excellent biochemical markers for congenital disorders of glycosylation (CDG) and also for chronic alcohol consumption. We have investigated the usefulness of typical "iron speciation" strategies to develop a method of enough resolution and sensitivity to unable the determination of individual Tf–Fe glycoforms. The method is based on high performance liquid chromatography (HPLC) coupled on-line with ICP-MS to determine ^{56}Fe and ^{57}Fe. This allowed the straightforward detection of six Tf glycoforms in healthy human serum after adequate iron saturation. Intact serum Tf glycoforms analysis by MALDI-TOF and by ES-Q-TOF were used for identification, but isotope dilution analysis using ^{57}Fe isotope will be described for absolute and accurate Fe-Tf isoforms determinations as biomarkers of more common parameters related to alcoholism and imbalances of iron homeostasis.

Another most important PTM of proteins, phosphorylation, determines the activity, subcellular localization, signalling potential, turnover and interactions of a given protein with other proteins, DNA or bioligands. Thus, phosphoproteomics is now an extense and active field of research demanding new ideas for phosphoprotein or phosphopeptide quantifications. The use of element-specific ICP-MS detection of phosphorus and capillary HPLC-ICP-MS analysis of proteins tryptic digests will be described. The high accuracy (around 2%) and precision attained using just a single reference phosphorous containing compound [Bis(4-nitrophenyl) phosphate] for calibration of all phosphopeptides of a tryptic digest of the protein make this strategy ideal for the investigation of small quantitative protein changes in functional and temporal studies involving signalling via phosphorylations, as it will be demonstrated for caseins (1).

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