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QUANTITATIVE ANALYSIS OF PROTEIN GLYCATION IN CLINICAL SAMPLES

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Non-enzymatic glycation is one of the post-translational modifications (PTMs) less frequently studied. An innovative approach for quantitative analysis of glycated proteins (GP) in clinical samples (serum, plasma and red blood cells) is here presented. It is based on relative quantitation of samples between two glycation states by differential labeling with light and heavy glucose ($^{12}C_6$- and $^{13}C_6$-glucose). Then, both sets of samples are pooled to carry out analysis by a shotgun proteomics workflow. This consists of in-solution digestion with endoproteinase Glu-C, selective enrichment of glycated peptides by boronate affinity chromatography (BAC), and analysis by RP-LC-ESI-MS/MS with an Orbitrap® mass analyzer (MS2 HCD higher energy collisional dissociation and data-dependent MS3 operation modes). A similar labeling efficiency has been observed with both isotopic glucose forms under the same operating conditions, which is essential for the applicability of the method. The identification and quantitation of GP is possible as the resulting peptides provide doublet signals in MS (labeling with light and heavy glucose) with a mass shift of +6, +3 or +2 Da depending on the peptide charge. With this methodology, it was possible to identify different GP such as serum albumin (with the five preferred glycation sites), immunoglobulins, haptoglobin, serotransferrin, complement C-3 and C-8 precursors, α-2-HS-glycoprotein or apolipoprotein A-1. These proteins are representative targets to compare between samples with different glycation states. This approach has also been applied to the analysis of clinical samples after depletion of more concentrated proteins. Further research is focused on the capability of the method to monitor the concentration of GP as well as to predict new potential targets for glycation. This can be especially interesting because it could be applied with prognosis/diagnosis purposes linked to pathological disorders related to glycemic control.