Strategies for proteomic analysis of blood glycated proteins

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Among post-translational modifications (PTMs) of proteins, non-enzymatic glycation is one of the less frequently studied in proteomics. Glycated proteins are formed by a non-enzymatic reaction between reducing carbohydrates (e.g., glucose, fructose, ribose or derivatives such as ascorbic acid) with amino groups located in N-terminal position or in lysine and arginine residues (Figure 1) [1]. Analytical guidelines required to succeed in the characterization of glycated proteins at qualitative levels have been recently proposed [2-4]. It involves the identification of glycated proteins as well as the elucidation of sugar attachment sites. Nevertheless, there is a demand for quantitative proteomic strategies that could be potentially applied for glycation assessment. One of the areas that could benefit from these strategies is the clinical field due to the crucial role of glucose as an energy source in humans, being the main circulating sugar and, thus, the most relevant molecule in terms of protein glycation. In fact, there are clinical evidences relating glycation and pathological conditions associated to hyperglycaemia such as diabetes mellitus, renal failure or aging [5-7].

A set of innovative approaches for analysis of glycated proteins is here presented by application to blood samples [8]. Qualitative analysis was carried out by tandem mass spectrometry (MS) after endoproteinase Glu-C digestion and boronate affinity chromatography (BAC) for isolation of glycated peptides. For this purpose, two MS operational modes were used: HCD-MS^2 and CID-MS^3 by neutral loss scan (monitoring two selective neutral losses typical of peptides modified by glucose attachment: 162.05 and 84.04 Da). Quantitative analysis was based on the labelling of proteins with $^{13}C_6$-glucose incubation in order to evaluate the native glycated proteins labelled with $^{12}C_6$-glucose. As glycation is chemo-selective, it is exclusively occurring in potential targets for in vivo modifications. This approach, named Glycation Isotopic Labelling (GIL), enabled to differentiate glycated peptides labelled with both isotopic forms resulting from enzymatic digestion by MS (6 Da mass shift per glycation site) as shown in Figure 2.

With two different purposes, this strategy has been applied to human plasma and lysates from

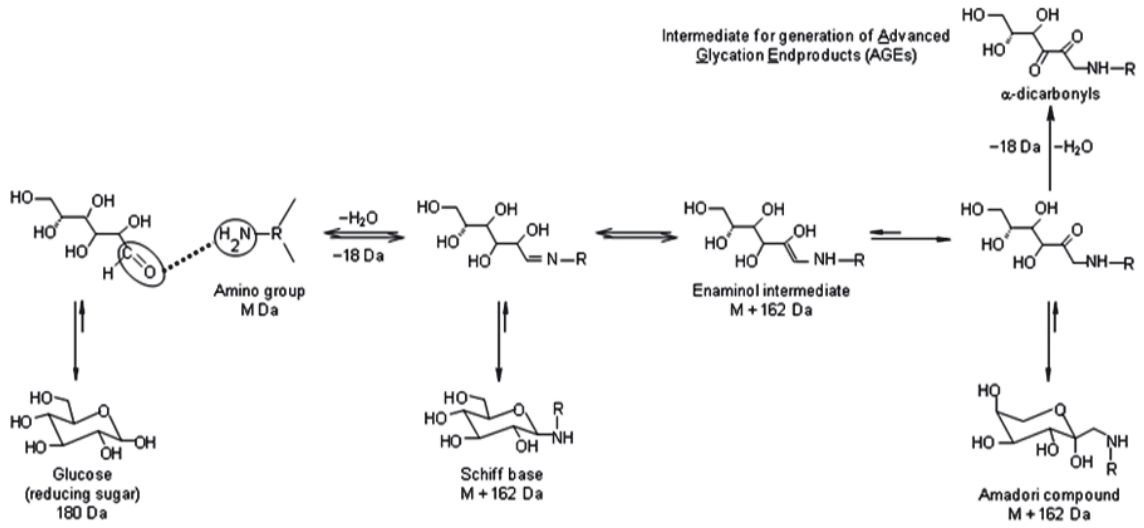

Figure 1. *Scheme of the glycation process (Maillard reaction).*

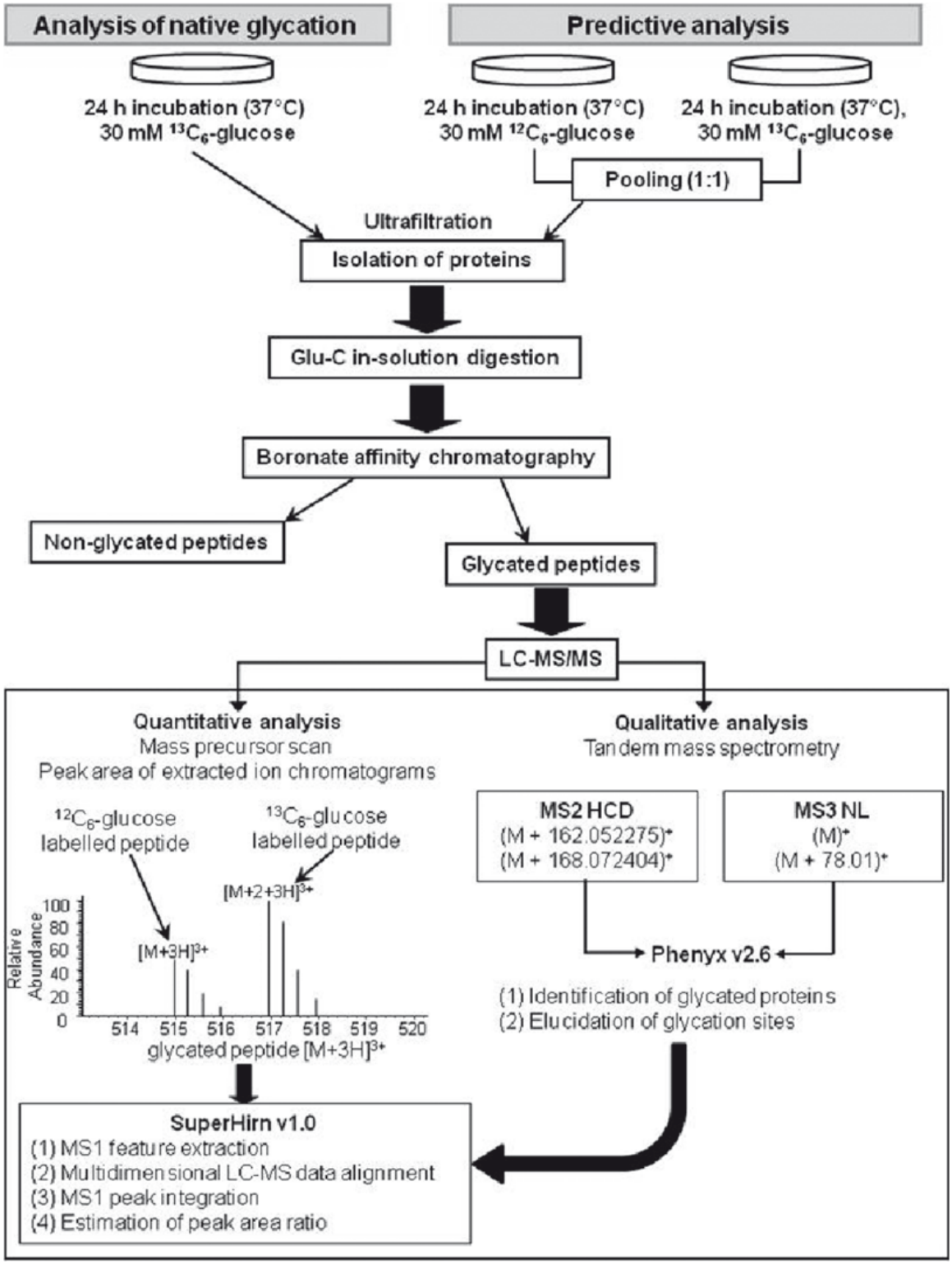

Figure 2. *Bottom-up proteomics workflows used for quantitative analysis of glycated proteins showing the labelling with light and heavy glucose.*

erythrocytes. The first one was based on the characterization of the native glycated proteome, incubating the clinical samples with $^{13}C_6$-glucose prior to analysis with the conventional proteomics workflow exposed above. Fifty glycated proteins with identification of 161 sugar attachment positions were detected in the analysis of human plasma. Concerning red blood cells, 20 glycated proteins with identification of 54 glycation sites were detected without any protein pre-fractionation step. These proteins are representative targets to compare between samples with different glycaemic states and monitor the glycaemic control. The second task was focused on the assessment of glucose stimuli at different concentrations (0, 5, 10, 30 and 50 mM glucose) in human plasma. For this purpose, five plasma aliquots were independently incubated with 0, 5, 10, 30 and 50mM $^{12}C_6$-glucose for 24 h and, subsequently mixed with a plasma aliquot incubated with 30mM $^{13}C_6$-glucose for 24 h. Proteomic analysis of these pools has revealed additional information about the biological effect of different hyperglycemia conditions and the kinetic of glycation.

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