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Quantitative Analysis of Glycated Proteins[†]

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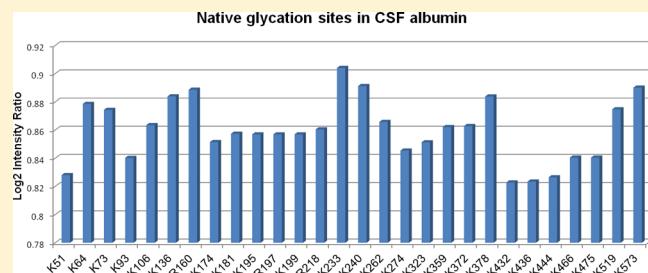
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Supporting Information

ABSTRACT: The proposed protocol presents a comprehensive approach for large-scale qualitative and quantitative analysis of glycated proteins (GP) in complex biological samples including biological fluids and cell lysates such as plasma and red blood cells. The method, named glycation isotopic labeling (GIL), is based on the differential labeling of proteins with isotopic [¹³C₆]-glucose, which supports quantitation of the resulting glycated peptides after enzymatic digestion with endoproteinase Glu-C. The key principle of the GIL approach is the detection of doublet signals for each glycated peptide in MS precursor scanning (glycated peptide with *in vivo* [¹²C₆]- and *in vitro* [¹³C₆]-glucose). The mass shift of the doublet signals is +6, +3 or +2 Da depending on the peptide charge state and the number of glycation sites. The intensity ratio between doublet signals generates quantitative information of glycated proteins that can be related to the glycemic state of the studied samples. Tandem mass spectrometry with high-energy collisional dissociation (HCD-MS₂) and data-dependent methods with collision-induced dissociation (CID-MS₃ neutral loss scan) are used for qualitative analysis.

KEYWORDS: nonenzymatic glycation, glucose, mass spectrometry, glycation isotopic labeling, hyperglycaemia, Amadori product, post-translational modification



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HISTORICAL BACKGROUND

Glycation is a common post-translational modification associated with the prevalence of glucose as a source energy in cells. Glycated proteins are formed by nonenzymatic condensation between reducing carbohydrates (e.g., glucose and fructose as the main sugars) with amino groups predominantly located in lysine residues and, less extensively, in arginine residues or N-terminus positions of proteins. The mechanism of the glycation process (Maillard reaction) is illustrated in Figure 1 with glucose as the sugar. The reaction between the reducing sugar and the amino group is initiated with the reversible formation of an adduct known as Schiff base by conversion of the aldehydic carbon–oxygen double bond of the sugar to a carbon–nitrogen double bound with the amine. The Schiff base is thermodynamically unstable in relation to the equilibrium cycled pyranose or furanose form. Therefore, the Schiff base is rapidly converted into an enaminol intermediate by rearrangement and, subsequently, to a ketoamine compound named Amadori compound. It is worth mentioning the biological differences between glycation and glycosylation, apart from the latter being

catalyzed by glycosyl transferase and occurring in specific protein side chains (mainly N-linked asparagine and O-linked serine and threonine). While glycosylation is involved in a diverse variety of essential and nonessential tasks, glycation is an undesired modification that causes impairment of protein function by structural damage or, at long-term, by formation of advanced glycation end-products (AGEs) that alter the structure of proteins. This defective modification is one of the mechanisms involved in hyperglycaemia and derived disorders.^{1–3}

A few studies have been reported for analysis of glycated proteins emphasizing the relevance of two particular steps of the analytical process:^{4–7} enrichment of glycated peptides and/or proteins and detection/identification based on mass spectrometry methodologies. Enrichment at peptide/protein level is crucial to allow detection in biological samples due to the low concentration of the glycated form of the proteins. Concerning the mass spectrometry analysis, the utilization of ion traps and Orbitrap mass analyzers seems to be the preferred option for characterization of this post-translational modification.

Here, we present the approach developed in our laboratory to analyze glycated proteins with identification of sugar attachment

[†]This Tutorial is part of the International Proteomics Tutorial Program (IPTP15).

Published: January 13, 2014

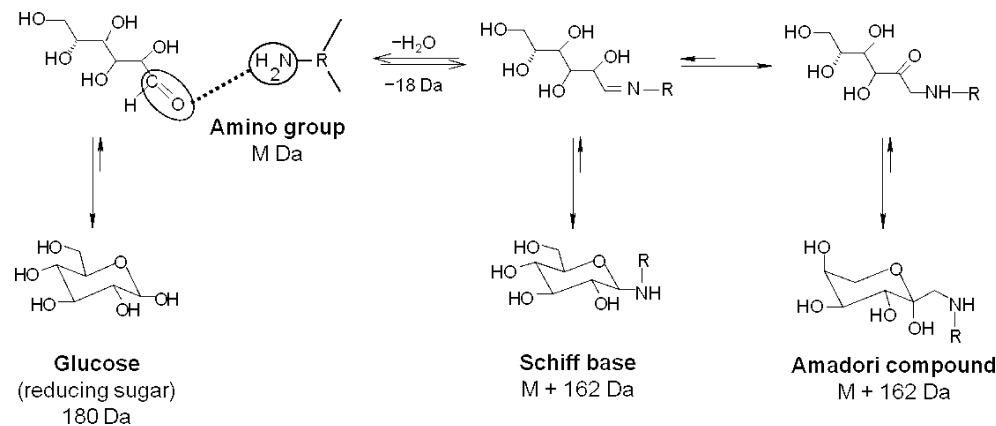


Figure 1. Mechanism of the nonenzymatic glycation of proteins based on the Maillard reaction. The amino group of the protein reacts with the reducing sugar through a reversible process to form the Schiff base, which is thermodynamically unstable. The Schiff base gives rise to a relatively stable ketoamine compound termed Amadori compound. Further, the Amadori compound can lead to a variety of carbonyl compounds, which act as propagators to the formation of a variety of heterogeneous structures irreversibly formed and commonly known as advanced glycation end-products (AGE).

sites in complex biological samples such as cellular extracts or body fluids. Our approach, schemed in Figure 2, is based on a protocol (detailed in Supporting Information) involving *in vitro* labeling of target glycation sites present in a biological sample with an isotopic $^{13}\text{C}_6$ -sugar (glucose as the main sugar) to pinpoint the detection of native glycated peptides in mass spectrometry precursor scanning. Target glycation sites are labeled through a chemo-selective process under physiological conditions to avoid sample alteration. Once marked proteins are digested, a mixture of peptides is obtained among which glycated peptides are a minor fraction. Isolation of glycated peptides is selectively attained with boronate affinity chromatography (BAC) to eliminate the redundant nonglycated fraction as an enrichment step prior to liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis. Structural assignment of glycated peptides and identification of modification sites are performed by combination of two MS operational modes: MS2 with high-energy collision dissociation (HCD) and MS3 with collision-induced dissociation (CID) in neutral loss scanning mode so as to enhance the detection coverage. This highly sensitive and selective approach is specially suited to characterize the glycated pattern of proteins, cells, tissues and physiological fluids, allowing not only the identification of glycation sites but also their relative quantitation.

BASIC CONCEPTS

Considerations for Biological Samples

As previously mentioned, the protocol can be applied for analysis of physiological fluids as well as cell lysates such as plasma and red blood cells. In any case, collection, processing and storage of biological material from volunteers and/or patients should be carried out following GLP. In the case of blood samples, the SOP described in the Supporting Information can be used.

In Vitro Labeling of Proteins by Incubation with $^{13}\text{C}_6$ -Glucose

Direct identification of glycated proteins in biological samples characterized by a complex matrix such as plasma, blood hemolysate, cerebrospinal fluid and cellular and subcellular fractions is a difficult task.⁸ Consequently, most glycation studies have been supported on *in vitro* experiments to obtain information about glycation sites and structural modifications of interest for *in vivo* situations.^{9–12} In this methodology, labeling with $^{13}\text{C}_6$ -glucose is a crucial step to favor identification of

native glycated proteins in experiments involving MS precursor scanning due to the detection of doublet signals for each glycated peptide. Evidently, this labeling is a chemo-selective process; in other words, only potential glycation targets will be marked isotopically. With this hypothesis, each glycated peptide provides a doublet signal in mass precursor scanning, which enhances the detection confidence of the approach. Additionally, the labeling step is a normalization operation that enables relative quantification of the glycation level of biological samples obtained under different conditions. Glycation isotopic labeling is supported on high reproducibility estimated by a low coefficient of variation for analytical replicates (<15%, data not published), which ensures quantitative capability of the overall method.

Labeling with $^{13}\text{C}_6$ -glucose is carried out under physiological conditions (pH and temperature) to avoid sample degradation. After incubation, samples can be stored without chemical alteration for months. This fact has been demonstrated by analysis of proteins from human hemolysate stored after glucose incubation.

Enzymatic Digestion of Glycated Proteins

Similarly to other post-translational modifications, enzymatic digestion is one of the most critical steps to ensure a high selectivity level in the identification of glycation sites. Mass spectrometry-based proteomics most of the time involves enzymatic degradation of proteins to peptides by trypsin hydrolysis. This protease is characterized by a high cleavage specificity and efficiency and is stable under a wide variety of conditions. Most importantly, cleaving C-terminal to arginine or lysine residues leads to peptides in the preferred mass range for effective fragmentation in tandem mass spectrometry (MS/MS) and places the highly basic residues at the C terminus of the peptides. This generally results in informative high mass y-ion series and makes tandem mass spectra more easily interpretable. However, the presence of glycation residues alters the digestion pattern of enzymes cleaving at lysine or arginine residues.⁸ For this reason, trypsin is not the suited enzyme for analysis of glycated proteins due to the generation of missed cleaved peptides.

The enzyme used in the protocol described here is endo-proteinase Glu-C that cleaves after glutamate residues but also can cleave after aspartate residues which is kinetically less favored than after glutamate residues (~100–300 times). Buffer composition strongly affects the specificity of Glu-C. In phosphate buffers, both glutamate and aspartate residues are cleaved; however, in

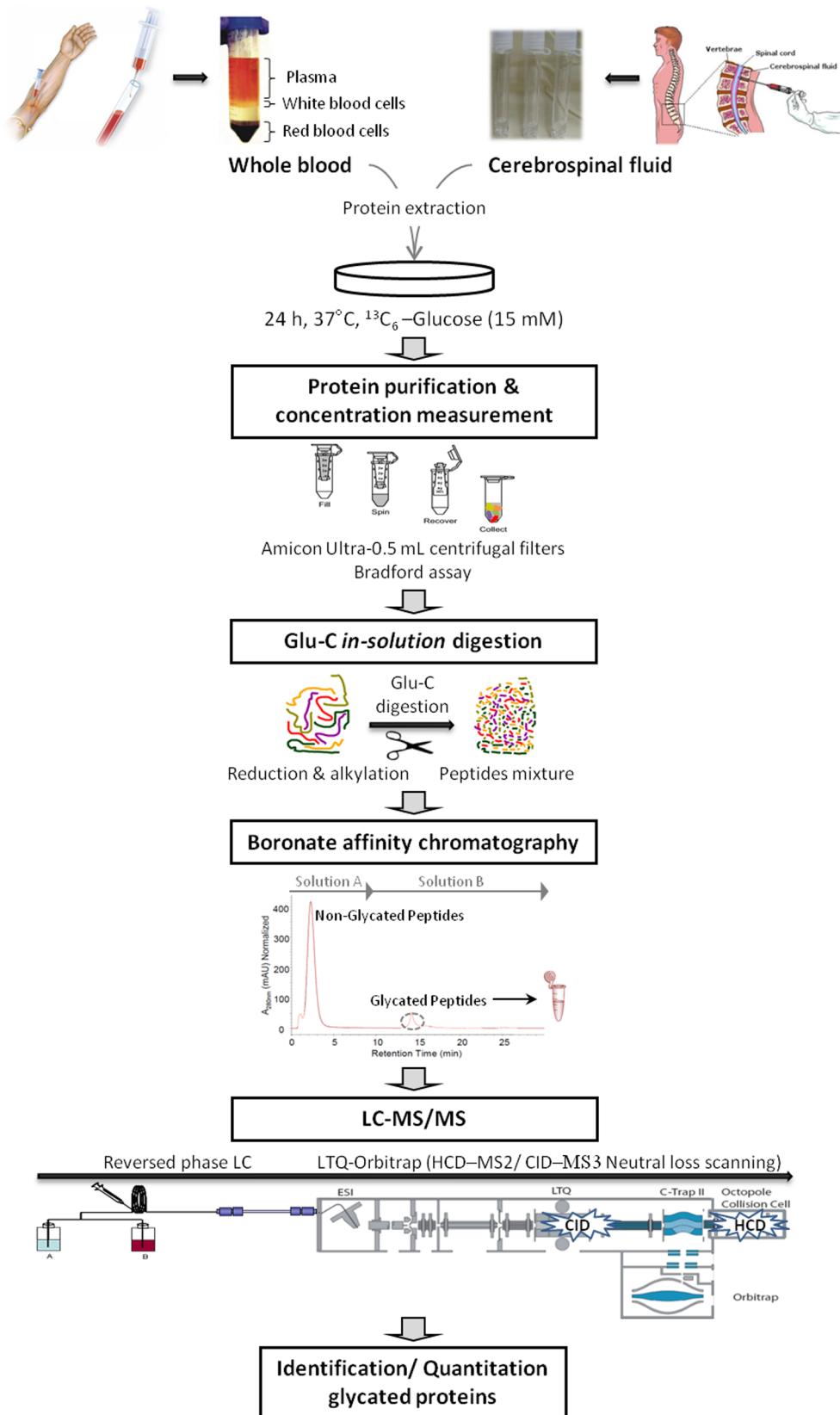


Figure 2. Scheme of the protocol proposed for comprehensive analysis of glycated proteins in human biofluids and cell lysates.

ammonium bicarbonate and ammonium acetate buffers (pH 4.0), only the glutamate residues are cleaved.¹³ Buffer selection could be an alternative to control this lack of selectivity. One other option is supported on the *in silico* detection of half-cleaved

peptides or by selection of enzymatic digestion pattern in phosphate buffers.

Since glycation is not affecting this amino acid residue, enzymatic digestion patterns for glycated and nonglycated proteins are very

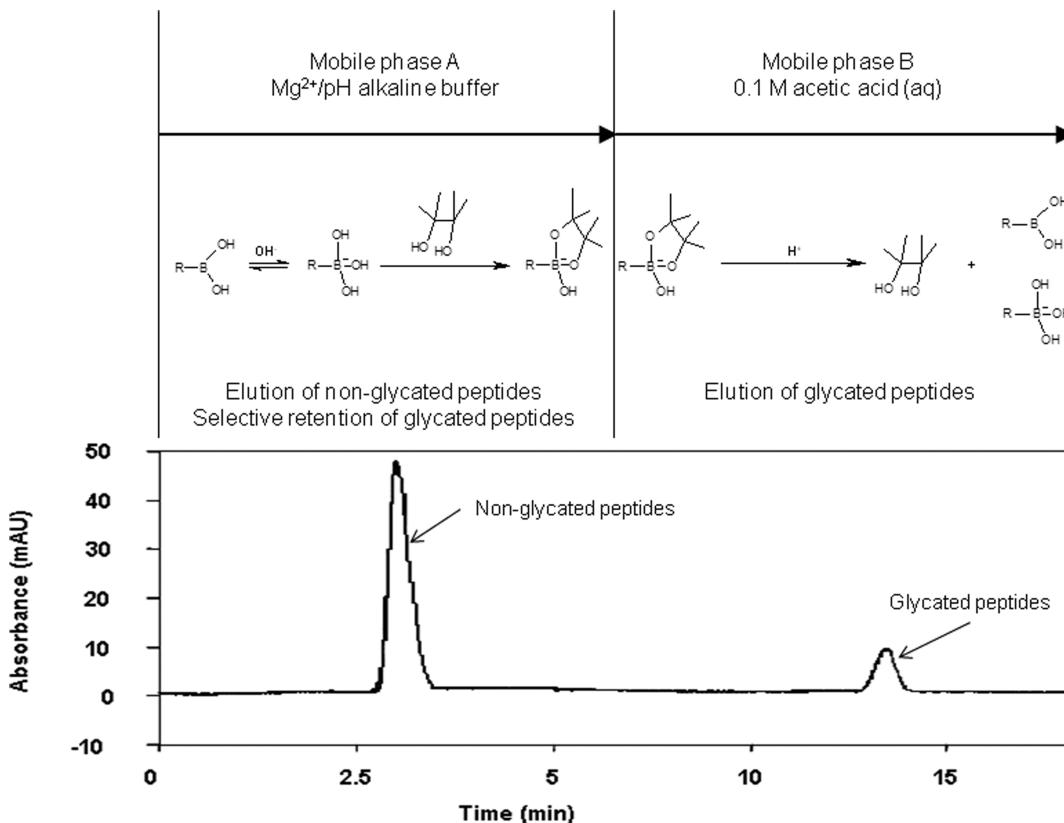


Figure 3. Boronate affinity chromatography separation of glycated peptides released after Glu-C digestion of proteins from euglycemic human serum. The mechanism of the separation process is supported on two steps: (i) retention of glycated peptides in the BAC column using an alkaline solution as initial chromatographic mobile phase while nonglycated peptides are washed out and (ii) elution of glycated peptides using an acid solution as mobile phase B.

similar. Apart from that, this enzyme generates longer peptides when compared to trypsin due to the lower occurrence of glutamate residues in human proteins. These large peptides, detected at high charge states, can be fragmented to generate characteristic fingerprints that can be accurately measured using high-resolution mass spectrometers.^{14,15} It is worth mentioning that quantitative recovery of glycated peptides is ensured in the complete protocol since the involvement of peptides labeled with the isotopic [¹³C₆]-glucose standardizes the measurement. The protocol has been preliminary optimized both with synthetic peptides but also with mixtures of model proteins.¹⁶

Enrichment of Glycated Peptides

There are two main limitations in the analysis of glycated peptides with conventional bottom-up proteomics protocols. The first one is the low ionization capability of glycated peptides in mass spectrometry detection under standard operation conditions. The second limitation is also associated to sensitivity due to the reduced efficiency of the glycation process and, thus, the low concentration of the glycated form of the proteins present in the human organism as compared to nonglycated proteins. Therefore, the application of selective steps for isolation and/or enrichment of glycated peptides is mandatory.

Affinity chromatography based on the utilization of boronic acids as ligands is the technique selected in the proposed protocol to enrich glycated peptides. This alternative, with the acronym BAC, is based on the selective interaction between boronic acids and biomolecules including *cis*-diol structures under alkaline conditions.¹⁷ A glucose moiety possessing this *cis*-diol configuration is responsible for retention of glycated

peptides by esterification of the two hydroxyl groups of *diol* with the boronate ligands.

On the basis of the above-mentioned mechanisms, BAC is highly efficient for isolation and enrichment of glycated peptides. The stationary phase usually is composed of phenylboronic acid derivatives covalently attached to an insoluble, inert agarose matrix. When the sample containing glycated peptides is run through the boronate affinity support, they are retained on the gel, whereas the nonglycated species are washed away. For this purpose, UV absorption is measured with a spectrophotometry detection system. The bound glycated peptides can then be collected by elution with an acidic pH mobile phase such as 0.1 M acetic acid and analyzed without the massive interference of nonglycated peptides. As can be seen in Figure 3, the fraction corresponding to glycated peptides is clearly minor as compared to that composed by nonglycated peptides.

Mass Spectrometry Analysis

Mass spectrometry analysis is crucial for detection of post-translational modifications. Concerning mass analyzers, ion trap and Orbitrap analyzers seem to be especially well suited for the characterization of PTMs since they allow the structural elucidation of modified peptides based on the efficiency of a theoretical MSⁿ process.¹⁸ In the protocol presented here, the Orbitrap analyzer is used to take the benefit from their mass accuracy in the detection of precursor and product ions, which enables the easy detection of glucose cleavage in tandem mass spectrometry analysis. Two complementary approaches are combined to enhance the identification capability of the method. Both approaches are based on tandem mass spectrometry but they use a different activation mode for the peptide

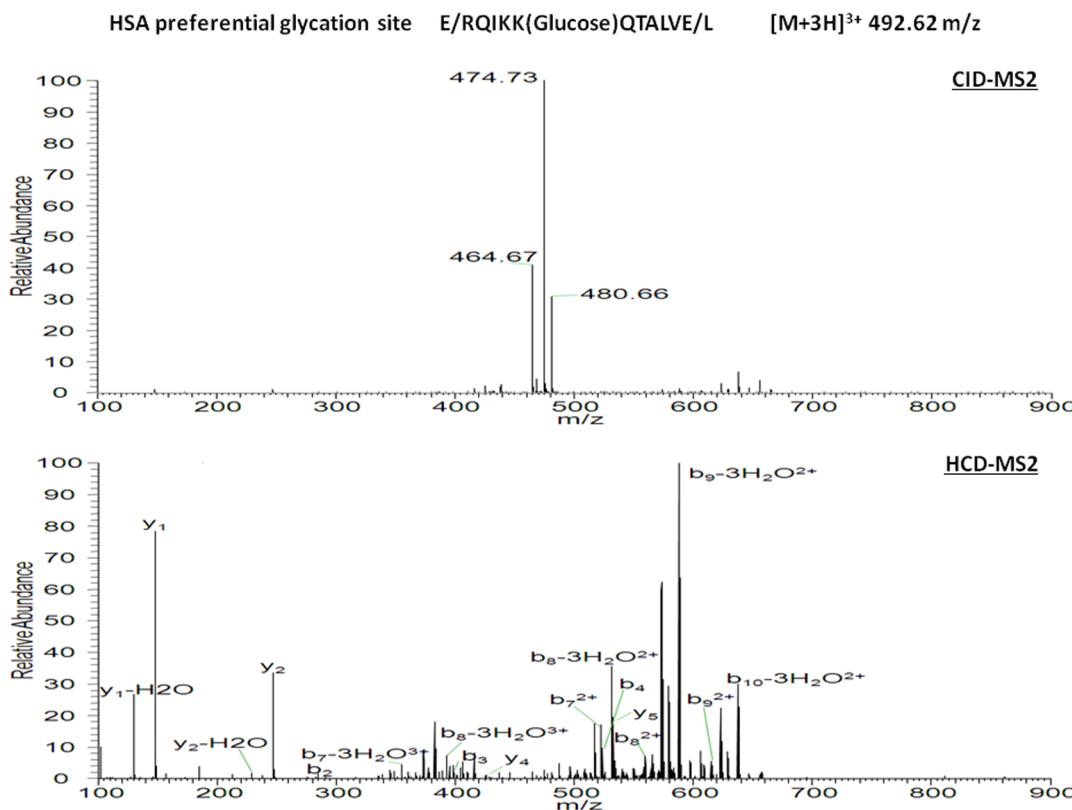


Figure 4. MS2 spectrum obtained by HCD activation of the glycated peptide containing the preferential modification site for human serum albumin. The most significant ions are indicated.

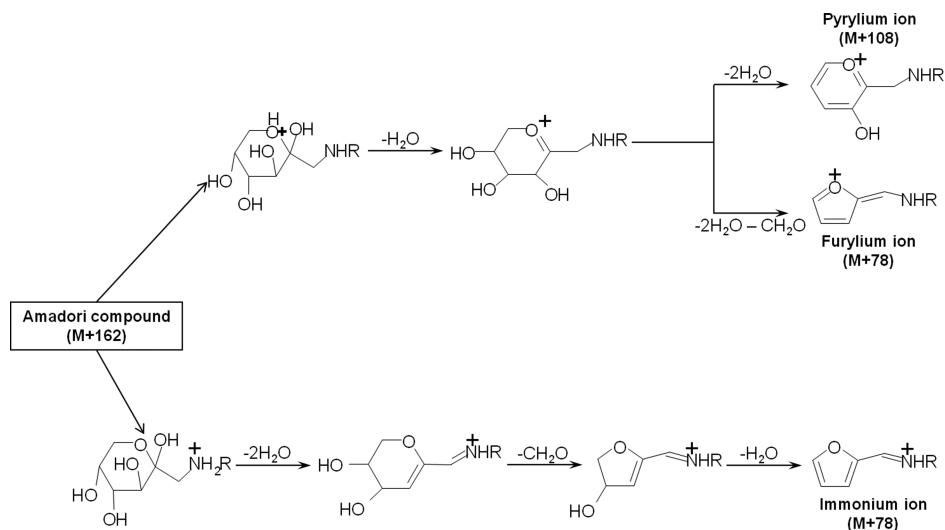


Figure 5. Fragmentation schemes of glycated peptides obtained by tandem mass spectrometry.

fragmentation. These are high-energy collisional dissociation in MS2 and collision-induced dissociation with data-dependent MS3 in neutral loss scan. HCD activation mode is characterized by its occurrence in the octopole collision cell annexed to the C-trap and nitrogen as collision gas. The use of nitrogen results in a more energetic fragmentation than helium-based dissociation occurring in CID. In addition, HCD is a fast activation mode as compared to CID, which may reach high vibrational energies per bond before dissociation of the target molecular ion.^{18–20} As a result, high-quality fingerprinting spectra are obtained as shown in Figure 4, which enhances the identification of long glycated peptides.

An additional benefit of HCD-MS2 is that immonium ions formed by MS2 activation can pinpoint the presence of glycated arginine and lysine.¹⁶ Thus, immonium-derived ions calculated for glycated arginine are at 207.124 and 237.135 Da (the most favored Arg immonium ion provides a signal at 129.114 Da, which is displaced to 291.166 Da with glucose attachment). Similarly, immonium-derived ions for glycated lysine are at 162.091 and 192.102 Da (84.081 Da as the most favored Lys immonium ion signal). The detection of immonium-derived ions for glycated Arg and Lys will aid users to assess the glycation level by observing the ion signals intensity. In addition, the same

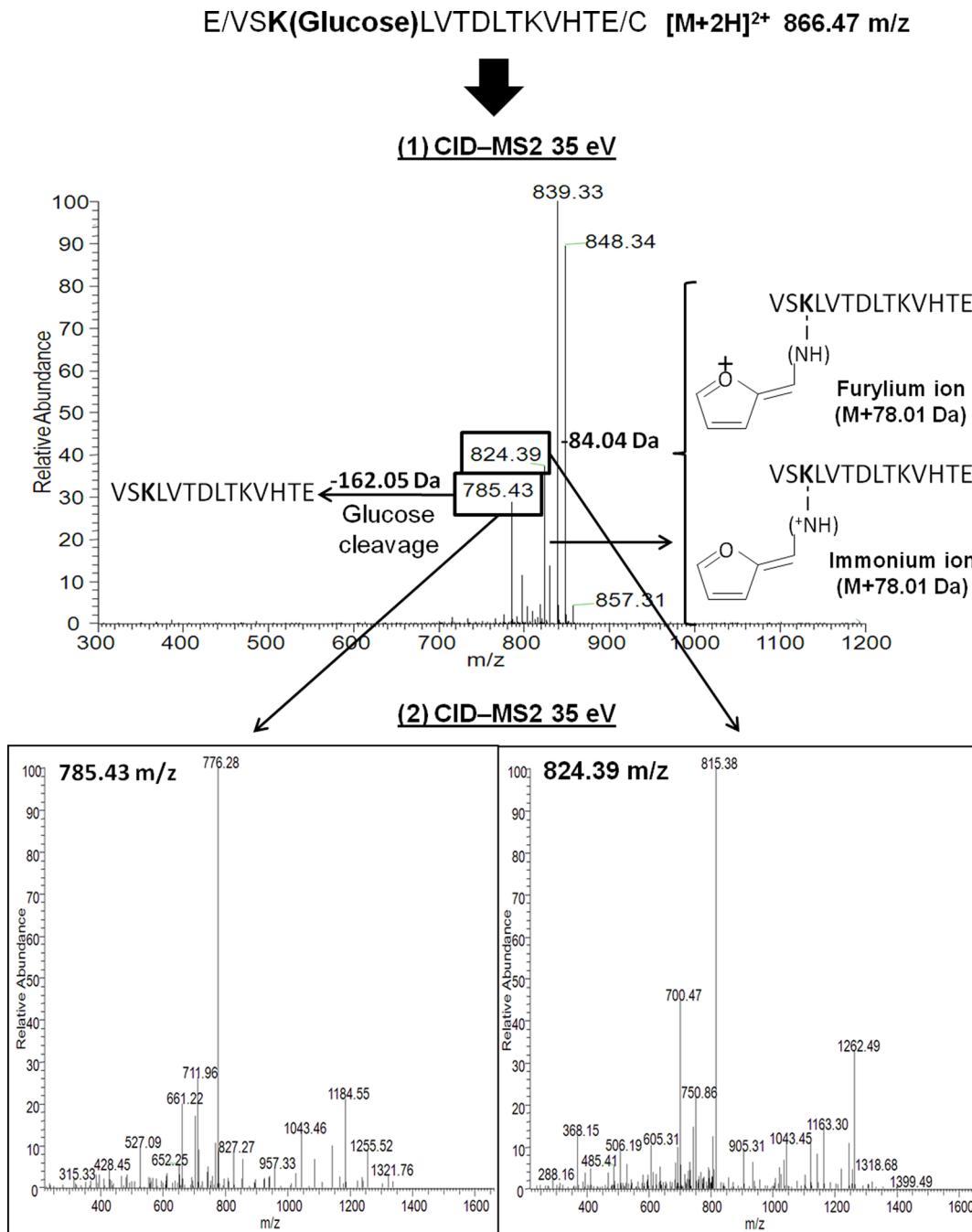


Figure 6. MS2 and MS3 spectra obtained by CID with a data dependent method based on neutral loss scanning. The example corresponds to a glycated peptide detected in human serum albumin.

strategy can be applied to estimate other immonium ions, which correspond to preferential glycation sites in specific proteins.

Analysis in MS2 is complemented by MS3 in neutral loss scanning. This process can be explained with the aid of Figure 5 that shows the different MS fragmentation pathways of glycated peptides as described in the literature.^{21,22} The mechanism of the MS3 neutral loss scanning is initiated by activation of the precursor ions in a first step. If the precursor ion corresponds to a glycated peptide, detected neutral losses make reference to the dehydration of up to three water molecules (18.01, 36.02, and 54.03 Da) to generate pyrilyium ion, dehydration with additional loss of a formaldehyde to form the furylium and immonium ions (84.04 Da), and the cleavage of the glucose moiety (162.05 Da).

After this fragmentation, only ions obtained by loss of 84.04 and 162.05 Da are isolated in the ion trap for a second fragmentation, which provides representative MS3 spectra for identification tasks. Ions formed by the other neutral losses (18.01, 36.02, and 54.03 Da by loss of water molecules) are excluded because they do not generate MS3 spectra with enough quality for identification. Since these ions still present labile parts in their structure, the formed MS3 spectra are similar to CID-MS2 spectra of glycated peptides, as shown in Figure 6.^{4,16}

According to the fundamentals of both operational modes, HCD-MS2 seems to be especially suited for detection of long glycated peptides (10–40 residues), most of which are formed by Glu-C enzymatic digestion, while CID-MS3 favors detection of

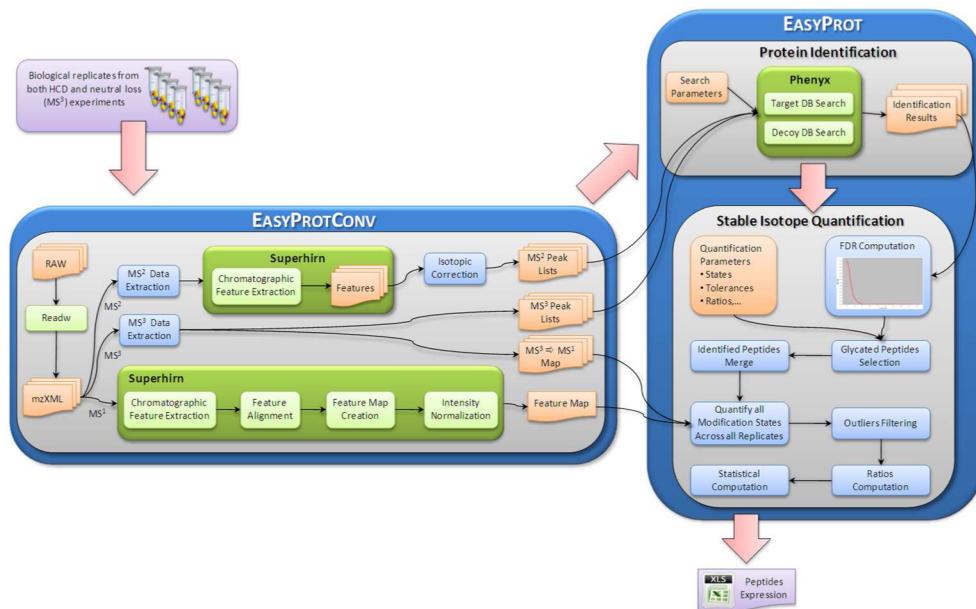


Figure 7. Scheme of the protocol proposed for treatment of raw data files applied to the qualitative and quantitative analysis of glycated proteins.

short glycated peptides. Therefore, the combination of both modes is justified by complementary detection of modified peptides.

Data Analysis: Identification and Quantitation of Glycated Proteins

A new stable isotope quantitation module was implemented in the EasyProt²³ platform to characterize and quantify proteins carrying glycated post-translational modifications. Two different approaches, one dedicated to HCD experiments and one based on neutral loss experiments, were developed and integrated into the EasyProt platform. The quantitation of glycated peptides is based on MS1 data while the identification of peptides is based on MS2 and MS3 data. The whole quantification workflow requires three steps which are described in the next paragraphs and illustrated in Figure 7.

Data File Preparation. The first step is to create the peak lists required by the peptide and protein identification engine. RAW data files are acquired on a Thermo LTQ Orbitrap XL. For HCD experiments, MS2 data originating from multiple replicates are converted to the MGF peak list format and then submitted to EasyProt for protein and peptide identification. Similarly, multiple replicates from neutral loss experiments fragmented in MS3 are converted to MGF and submitted for identification. The next step is to detect, extract and align chromatographic peaks from multiple MS1 replicates in order to create global feature maps. Quantitative values are obtained by integrating ion abundance (through m/z and retention time using the Superhirn²⁴ software) under the chromatographic curve for a given detected chromatographic peak. These feature maps, one for HCD analyzes and one for neutral loss experiments, contain quantitative data extracted from ion abundance, which is later to be matched to identified peptides. Furthermore, because the neutral loss experiment features peak lists based on MS3 spectra instead of MS2 spectra, an additional step is required. Indeed, later on it is necessary to lookup MS1 data based on peptides identified from MS3 data. To achieve this goal, an indirection map allowing to lookup MS1 data from MS3 spectra is created along the global feature map.

Protein Identification. The second step is the identification of peptides and proteins from peak lists generated in the first step described previously. Each identified peptide is characterized by a sequence, a charge, a mass (m/z) and a retention time according to its elution properties. For a given experiment (HCD or neutral loss), assessing the relative quantification of glycated peptides requires combining or matching identified peptides with the global feature maps generated during the first stage. This matching first requires locating all glycated peptides. To do so, only peptides carrying mass shifts corresponding to light or heavy glycation modification forms are kept. Then, each glycated peptide is looked up in the feature map according to their m/z and charge to locate the corresponding quantitative value (i.e., chromatographic feature). Usually, only one form of a peptide is identified, either the light or the heavy form, but almost never both at the same time. Because of this, it is necessary to find the quantitative value of the missing form. The quantification of the missing peptide is implemented by searching the corresponding m/z at a similar retention time. Because of their physicochemical properties, both forms elute around the same time. Since one peptide of the pair is usually identified, its charge, m/z and retention time are known. It is therefore possible to deduce these very parameters for the missing peptide form since the retention time between both forms is similar and m/z values are shifted by the mass shift of the difference between the light and heavy modifications. In case of neutral loss experiments, an additional lookup through the indirection map generated previously is required to go from MS3 to the global feature map storing MS1 quantitative values. Hence, for a neutral loss experiment, the mapping becomes the following: identified peptide (MS3) to indirection map to MS1 quantitative values (from the global feature map).

Glycated Peptide Quantification. The last step is the combination of quantitative values with regard to assessing the expression of each glycated peptide. Doublet signals representing precursor abundance for light/heavy glycated forms are computed for every peptide identified in at least one of the replicates. To increase the reliability of our LC–MS-based quantitative approach, quantified peptides present in less than two replicates

are discarded. Outlier removal is based on filtering out values falling outside (typically 95%) the distribution of abundances per peptide. Ratios are computed for each peptide and key statistical metrics are calculated, such as median, min, max, first and third quartiles. These statistical values as well as other relevant information such as the list of all quantified peptides from all replicates are then written to an Excel file.

■ WORKED EXAMPLES

The main application fields of this approach are to unravel specific aspects of hyperglycaemia effects and to evaluate the efficiency of normoglycaemia treatments. In this sense, the presented approach could be applied as a complementary tool to other studies previously reported for characterization of glycation in different pathologies such as diabetes.^{25–27} It is worth taking into account that the glycemic control is currently assessed in individuals with the reference test for measurement of glycated hemoglobin (HbA1c). However, the proposed strategy may also provide semiquantitative information to monitor the glycemic control of samples under different physiological states. Also, a perspective about the glycated profile of any biological sample under certain conditions can be obtained. These profiles can be correlated with the glycemic state of individuals. Recently, we have successfully applied this approach to the analysis of native

glycated proteins from different human blood components, including plasma and hemolysates.^{16,28} We have also identified native glycation by applying this approach to the human cerebrospinal fluid.²⁸ The approach has been used to propose a mass spectrometry strategy for the characterization of glycated proteins in the prognosis of diabetes and neurological disorders.^{28,29} Thus, this approach can be used to study the glycated proteins encountered in a complex biological medium (e.g., fluid, cells, organ, and tissue).

Assessment of the Native Level of Protein Glycation in Biological Samples

The glycemic state of individuals is currently evaluated with the test for measurement of glycated hemoglobin (HbA1c) concentration, which is a long-term indicator due to the erythrocytes lifespan (~120 days).³⁰ However, other measurements indicative of short-term glucose perturbations or indicative of glycaemia in other biofluids or tissues are required to establish the biological effect of glycation. More importantly, any protein can be theoretically glycated.

RAW data acquired from MS2-HCD and MS3 neutral loss experiments can give users significant information of the native background glycation in target samples. Due to the selectivity of immonium ions and the high accuracy of MS2 fragments with

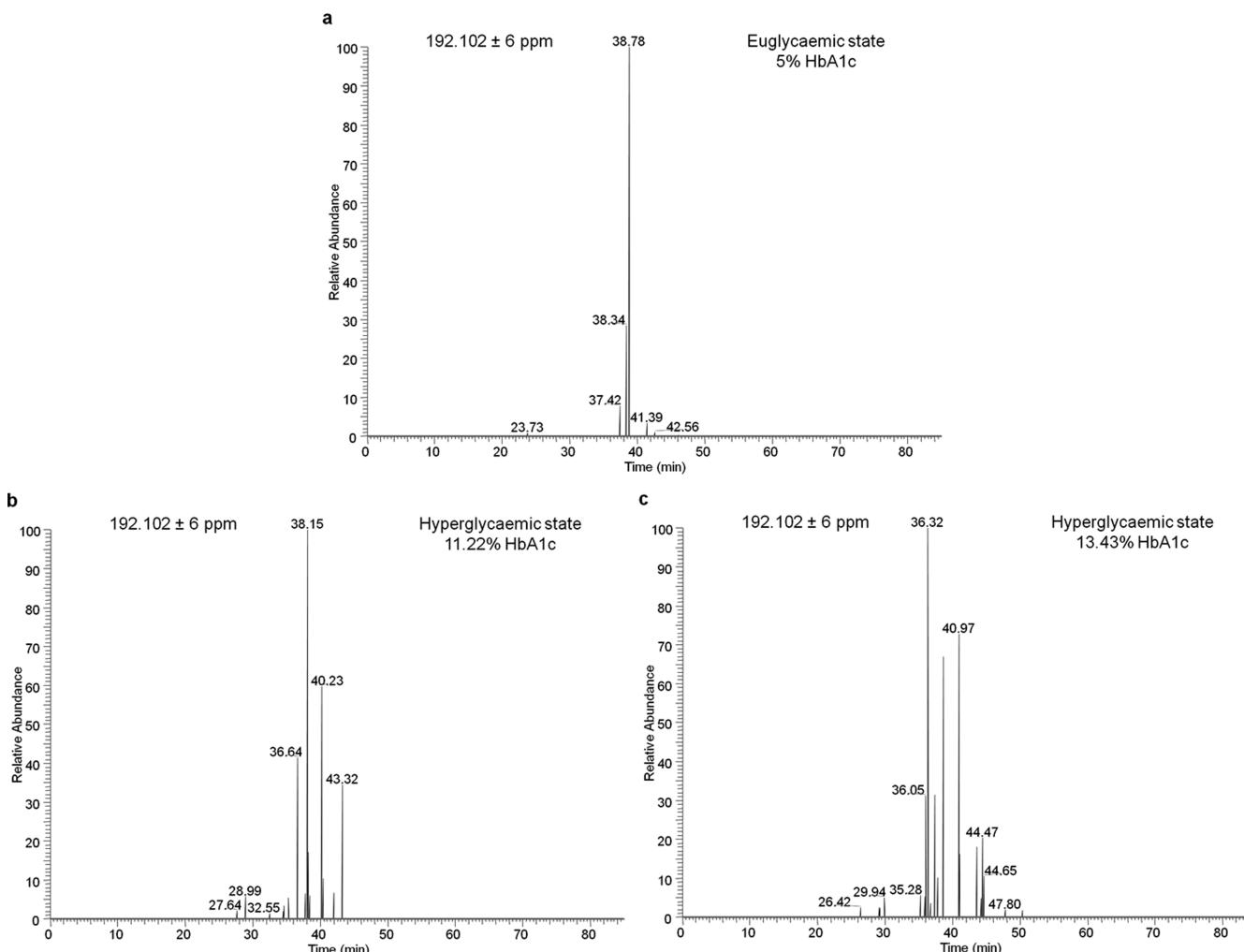


Figure 8. Native glycation profile of blood hemolysates from (a) euglycemic and (b and c) hyperglycemic individuals by monitoring extracted MS2 chromatograms for immonium ions of glycated lysine residues. The value marked for each signal (which should fit with one glycated peptide) corresponds to the chromatographic retention time expressed in min.

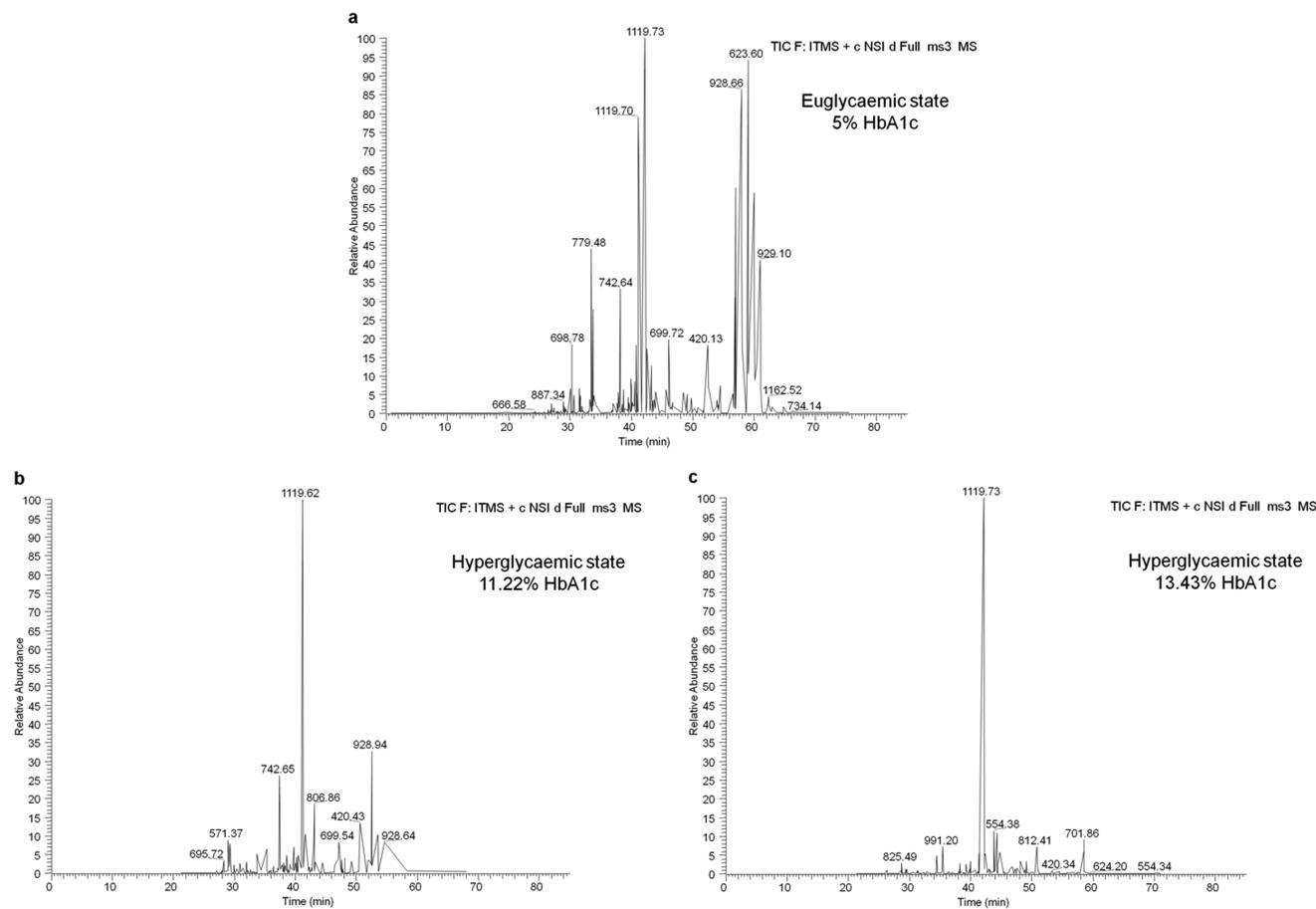


Figure 9. Native glycation profile of blood hemolysates from (a) euglycemic and (b and c) hyperglycemic individuals by monitoring total ion MS3 chromatograms obtained in neutral loss scanning mode. The value labeled for each signal corresponds to the m/z ratio of the base peak obtained in each MS3 spectrum.

Orbitrap detection, glycated peptides may be mapped by extracting ion chromatograms in MS2 scans. Figure 8 shows the extracted ion chromatograms in MS2 for the immonium ion estimated for glycated lysine residues (190.102 Da) provided by the analysis of blood hemolysates from euglycemic (5% HbA1c) and hyperglycemic (11.22 and 13.43% HbA1c) individuals. Each signal, labeled with chromatographic retention time expressed in min, should fit one glycated peptide. As can be seen, the relative abundance of glycated peptides increased considerably in the hemolysates corresponding to hyperglycemic states.

This background glycation can also be deduced by MS3 total ion extracted chromatograms acquired after neutral loss because of the high selectivity of the MS operational mode. The selectivity of this analysis is supported by the fact that only glycated peptides are reisolated in the ion trap for a second fragmentation step. Thus, this analysis can also be used to compare the glycemic state of individuals as shown Figure 9 with MS3 extracted chromatograms (labeled values correspond to the m/z ratio of the base peak obtained in each MS3 spectrum) provided by the analysis of blood hemolysates from euglycemic and hyperglycemic individuals. As can be seen, the profile of the chromatogram changes considerably for hyperglycemic individuals increasing in relative terms the signal that corresponded to the peptide containing the preferential glycation site of human hemoglobin located at the N-terminus site of the β chain (m/z 1119.7 ± 0.1 is the base peak obtained by MS3 fragmentation of the cited peptide).

The native glycation can be estimated in relative terms by using the quantitation approach based on the differential labeling with glucose to compare biological states. The estimation of the peak area ratio between the native and the *in vitro* glycated peptides can provide a global view about the glycemic state of a particular biological sample. This analysis also reports information about the preferential glycation sites for each detected protein as shown Figure 10 for human albumin detected in cerebrospinal fluid.

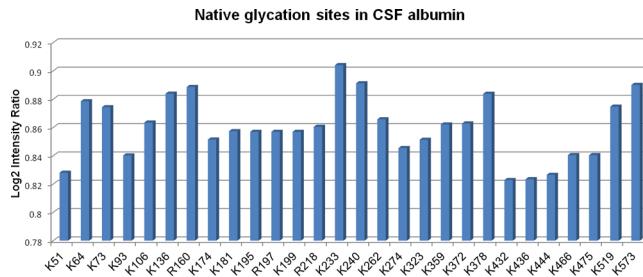


Figure 10. Glycation affinity of the different sites identified in human serum albumin detected in cerebrospinal fluid.

Predictive Analysis of Glycated Proteins

In addition to the analysis of native glycation, predictive analysis about the possible incidence of hyperglycaemia conditions over

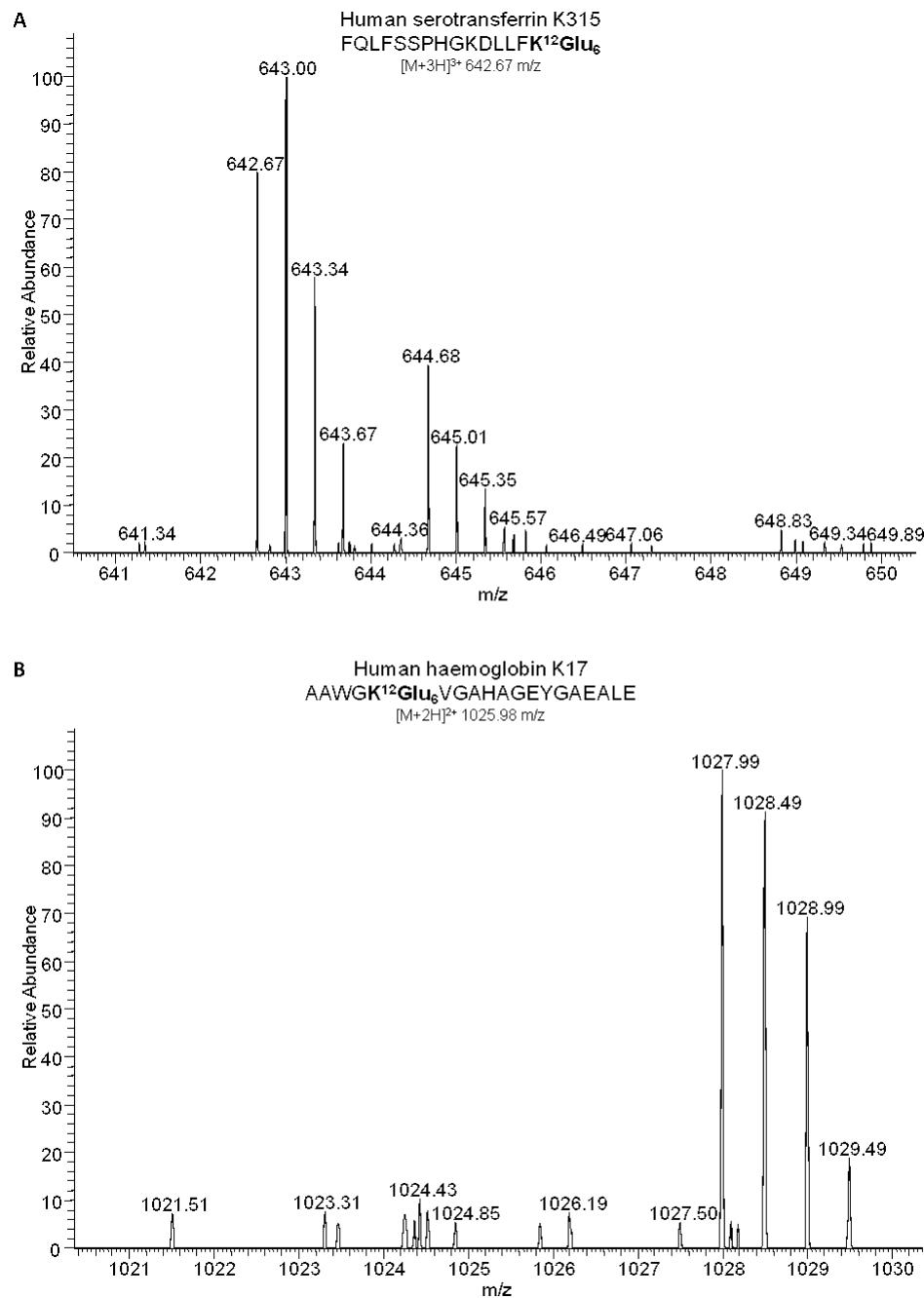


Figure 11. Prediction analysis of two glycated peptides from proteins present in human biofluids. (A) Serotransferrin peptide detected in human serum modified in position K315. (B) Hemoglobin peptide (α chain) detected in blood hemolysate modified in position K17.

target glycation sites can also be determined by current protocol. For this purpose, we have to consider the signals ascribed to peptides labeled with [$^{13}\text{C}_6$]-glucose taking as reference in this case the signal corresponding to the native glycated peptide. Additionally, new glycation targets can be identified if only the [$^{13}\text{C}_6$]-glucose-labeled peptide is detected and no doublet is found. Figure 11 illustrates a representative example of glycated peptides detected in human body fluids. As can be seen, in the first case (Figure 11A), we can obtain information about the behavior of a glycated peptide under prolonged hyperglycaemia conditions by measuring the signal of the [$^{13}\text{C}_6$]-glucose-labeled peptide, which is less intense than that observed for the native [$^{12}\text{C}_6$]-glucose labeled peptide. In the second case (Figure 11B), it is a nonglycated peptide

in native conditions but the [$^{13}\text{C}_6$]-glucose labeled peptide (m/z 1027.99) is detected as a result of the *in vitro* glucose addition.

CURRENT LIMITATIONS AND USEFUL WORKING LIMITS

The application of the described methodology provides qualitative and quantitative information in relative terms. Therefore, the main limitation is that no absolute quantitative information can be obtained. For this purpose, selected reaction monitoring (SRM) based methods with synthetic peptides as internal standards should be the preferred option. Nevertheless, absolute quantitation of a target glycated protein also demands for quantitation of the corresponding nonglycated protein, which would complicate development of methods addressing this issue.

An additional aspect is the absence of approaches coupling affinity chromatography based on boronate ligands and mass spectrometry. BAC separation is currently implemented as preparative chromatography in off-line configurations. Therefore, online separation of glycated peptides with subsequent MS detection would improve analytical features such as sensitivity, selectivity and precision.

FUTURE DEVELOPMENTS

The qualitative/quantitative approach presented here for analysis of glycated proteins in biological samples opens new expectations for characterization of the human glycated proteome. Hyperglycaemia, which causes negative effects on different tissues, is a conditioning factor promoting the nonenzymatic glycation of proteins in those sites kinetically favored. The glycated proteome is characterized by its dynamic profile which evolves qualitatively and quantitatively with unbalanced glucose concentration. With these premises, it is interesting to obtain profiles containing information of glycation sites as a function of hyperglycaemia level. As an example, the application of the method described here has revealed the identification of 50 glycated proteins in normoglycaemic plasma with detection of 161 glycation sites.¹⁶ The dynamism of the glycated proteome occurring under hyperglycaemia justifies the mapping of glycated proteins in different biological samples to understand modifications occurring owing to unbalanced glucose homeostasis.

ASSOCIATED CONTENT

Supporting Information

Supplementary PowerPoint presentation. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was initiated at the Biomedical Proteomics Research Group (BPRG), University of Geneva, supported by funds of the Swiss SystemsX.ch initiative (grant IPP-200N/ONN) and Fonds National Suisse (FNS) pour la recherche scientifique (Grant 31003A-134756), the Spanish Ministry of Science and Innovation (grants with reference 2007-0398 and RYC-2009-03921) and the Junta de Andalucía (grant FQM-2010-6420). We also thank those who have donated their CSF and blood for our studies.

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