Analytical Biochemistry 419 (2011) 81–87

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

The isotopic exchange of oxygen as a tool for detection of the glycation sites in proteins

Monika Kijewska, Piotr Stefanowicz *, Alicja Kluczyk, Zbigniew Szewczuk

Faculty of Chemistry, University of Wrocław, 50383 Wrocław, Poland

ARTICLE INFO

Article history:
Received 28 March 2011
Received in revised form 7 August 2011
Accepted 22 August 2011
Available online 27 August 2011

Keywords:
Isotopic exchange
^{18}O
Glycation
Amadori
Mass spectrometry

ABSTRACT

A nonenzymatic reaction of reducing sugars with the free amino group located at the N terminus of the polypeptide chain or in the lysine side chain results in glycation of proteins. The fragments of glycated proteins obtained by enzymatic hydrolysis could be considered as the biomarkers of both the aging process and diabetes mellitus. Here we propose a new method for the identification of peptide-derived Amadori products in the enzymatic digest of glycated proteins. The products of enzymatic hydrolysis of the model protein ubiquitin were incubated with $H_2{}^{18}O$ under microwave activation. We observed that at these conditions the Amadori compounds selectively exchange one oxygen atom in the hexose moiety. The characteristic isotopic pattern of Amadori products treated with $H_2{}^{18}O$ allows fast and convenient identification of this group of compounds, whereas nonglycated peptides are not susceptible to isotopic exchange.

The nonenzymatic posttranslational modifications of proteins are considered as key factors in the pathogenesis of a large number of disease states. The homocysteinylation [1] by homocysteine lactone is attributed to the cardiovascular diseases, the oxidative stress results in oxidation of methionine, nitration of tyrosine, and modification of lysine side chains by 4-hydroxy-2-nonenal (4-HNE)[1] formed by lipids peroxidation [2]. The glucose is less reactive; however, under physiological conditions, it slowly interacts with the amino groups of proteins forming products of Amadori rearrangement. The rate of formation of Amadori products correlates well with the average glucose concentration in blood; therefore, the glycated fragments of blood proteins are potentially useful markers of diabetes [3].

Recently, a remarkable effort has been made to characterize the glycation of plasma (serum) proteins using the proteomic methods [4]. Successful analysis of the extremely complex system such as the products of plasma hydrolysis usually requires both the method of preconcentration of the modified peptides and the method of their selective detection.

In recent articles, the products of enzymatic plasma hydrolysis were separated using a borate affinity column [5–7]. This procedure eliminates the nonglycated peptides, and the enriched sample could be further analyzed by the liquid chromatography–mass spectrometry (LC–MS) method [5,6]. The Amadori products were detected on the basis of their characteristic series of neutral losses [8–10]. The collision-induced dissociation (CID) spectra are poor in sequential information; therefore, the identification and sequencing of Amadori products may be supported by the electron transfer dissociation (ETD) method [11]. The electron capture dissociation (ECD) has also been presented as an efficient technique of glycated peptide sequencing [12].

It has been shown that combining the isotopic labeling and MS is a very efficient method for detection of glycation sites in proteins. In our recent article, the sample of the investigated protein was modified by the equimolar mixture of glucose and $^{13}C_6$ glucose [13]. A similar approach was reported independently by Priego-Capote and coworkers [14]. The isotopic labeling with ^{13}C glucose allows detection of Amadori products in complex mixtures formed by the enzymatic hydrolysis of glycated peptides and can be useful for verification of the data obtained by the tandem mass spectrometry (MS/MS) technique. On the other hand, this method is limited by the necessity of using isotopically labeled glucose for glycation at the stage of protein modification. It makes difficult generalization of the mentioned approach with respect to real clinical samples.

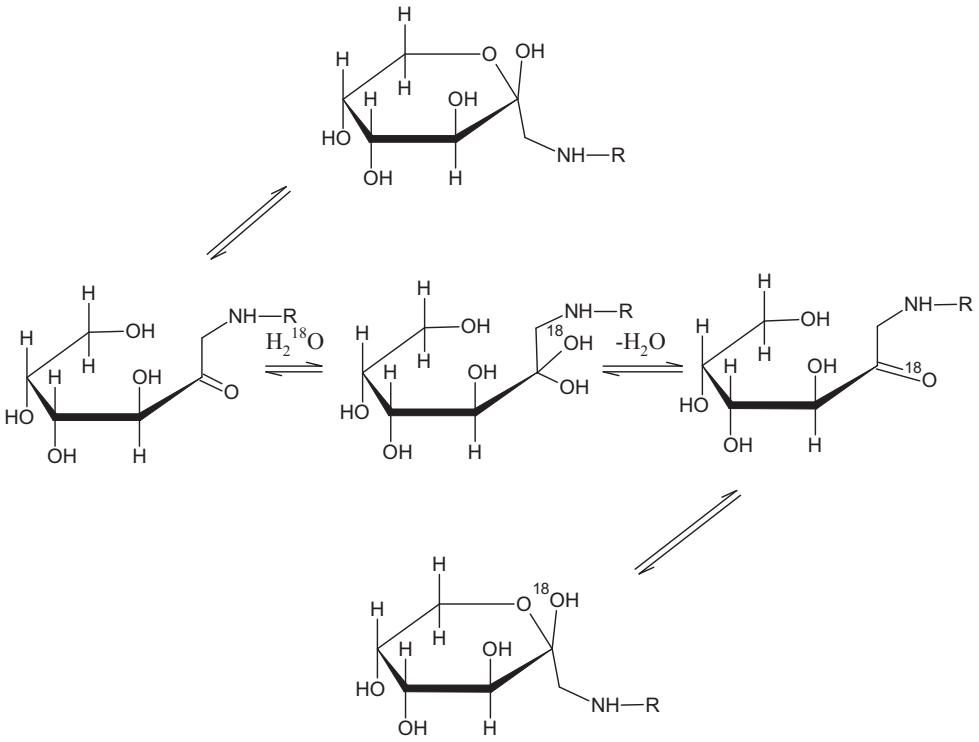
The structure of peptide-derived Amadori products in solution is dominated by cyclic furanose and pyranose forms [15]. However, in some cases the nuclear magnetic resonance (NMR) data indicate in the equilibrium the presence of the open-chain form (the hydrated keto form) [15]. This last form is susceptible to isotopic exchange in $H_2{}^{18}O$ by the reversible binding of an isotopically labeled water molecule (Scheme 1).

Our recent studies have proven that the glycated peptides incubated with $H_2{}^{18}O$ exchange selectively and almost quantitatively

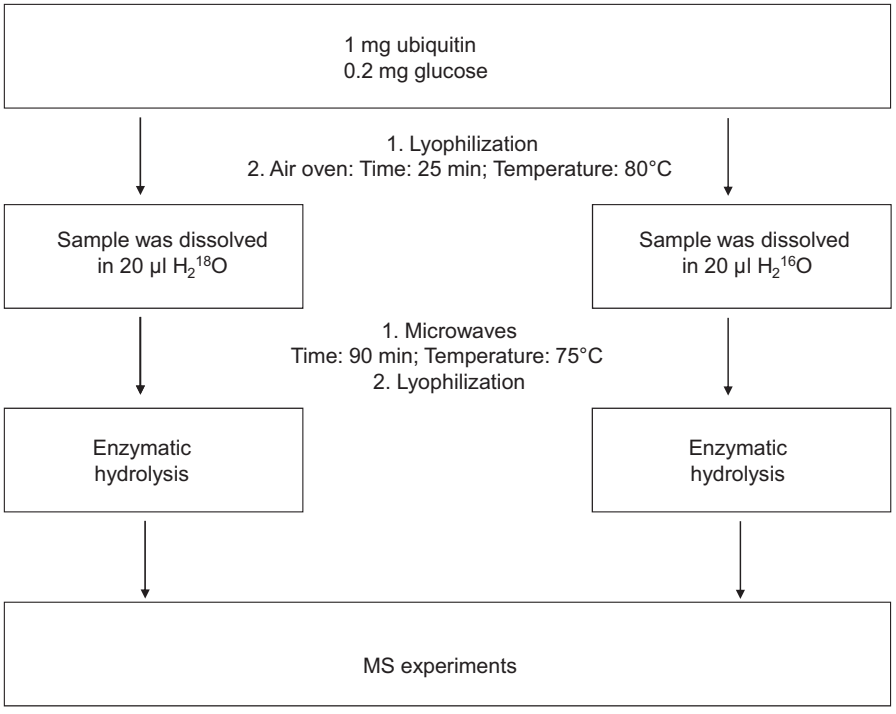
* Corresponding author.
E-mail address: stp@wchuwr.pl (P. Stefanowicz).

[1] *Abbreviations used:* 4-HNE, 4-hydroxy-2-nonenal; MS, mass spectrometry; CID, collision-induced dissociation; MS/MS, tandem mass spectrometry; ESI, electrospray ionization.

0003-2697/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2011.08.040



Scheme 1. Proposed mechanism of $^{16}O/^{18}O$ isotopic exchange of oxygen atom attached to the anomeric carbon atom in Amadori products.



Scheme 2. Microwave-activated $^{16}O/^{18}O$ isotopic exchange in glycated ubiquitin.

the oxygen atom attached to the anomeric carbon atom, resulting in an increase in molecular mass by 2 units per glycation site present in peptide treated with $H_2{}^{18}O$ [16]. The current article shows the model study on the possibility of detection of the glycation sites by the procedure combining the isotopic exchange and MS. The presented approach can be adapted for analysis of in vivo glycated proteins.

Materials and methods

Reagents

Ubiquitin (isolated from bovine blood cells) and pepsin were purchased from Sigma–Aldrich. $H_2{}^{18}O$ (97% ^{18}O atoms) was purchased from ISOTEC (Aldrich). D-Glucose was purchased from Eurochem.