Biochimica et Biophysica Acta 1840 (2014) 1712–1724

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Deciphering metal-induced oxidative damages on glycated albumin structure and function

Jennifer Baraka-Vidot [a,1], Giovanna Navarra [b,*,1], Maurizio Leone [b,c], Emmanuel Bourdon [a], Valeria Militello [b,c], Philippe Rondeau [a,**]

[a] Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO) EA 4516, Université de La Réunion, Faculté des Sciences, 15 avenue R. Cassin, BP 7151, 97715, Saint Denis Cedex and Plateforme CYROI, 2 Rue Maxime Rivière, BP 80 005, 97491 Sainte Clotilde Cedex, Reunion Island, France
[b] Università di Palermo, Dipartimento di Fisica e Chimica, Via Archirafi 36, Palermo, Italy
[c] Consiglio Nazionale delle Ricerche, Istituto di Biofisica, Via Ugo La Malfa 136, Palermo, Italy

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form 25 November 2013
Accepted 9 December 2013
Available online 29 December 2013

Keywords:
Glycation
Albumin
Protein aggregation
Spectroscopy
Light scattering
Metal

ABSTRACT

Background: Metal ions such as copper or zinc are involved in the development of neurodegenerative pathologies and metabolic diseases such as diabetes mellitus. Albumin structure and functions are impaired following metal- and glucose-mediated oxidative alterations. The aim of this study was to elucidate effects of Cu(II) and Zn(II) ions on glucose-induced modifications in albumin by focusing on glycation, aggregation, oxidation and functional aspects.
Methods: Aggregation and conformational changes in albumin were monitored by spectroscopy, fluorescence and microscopy techniques. Biochemical assays such as carbonyl, thiol groups, albumin-bound Cu, fructosamine and amine group measurements were used. Cellular assays were used to gain functional information concerning antioxidant activity of oxidized albumins.
Results: Both metals promoted inhibition of albumin glycation associated with an enhanced aggregation and oxidation process. Metal ions gave rise to the formation of β-amyloid type aggregates in albumin exhibiting impaired antioxidant properties and toxic activity to murine microglia cells (BV2). The differential efficiency of both metal ions to inhibit albumin glycation, to promote aggregation and to affect cellular physiology is compared.
Conclusions and general significance: Considering the key role of oxidized protein in pathology complications, glycation-mediated and metal ion-induced impairment of albumin properties might be important parameters to be followed and fought.

1. Introduction

Protein alterations represent a growing interest in biophysical and biochemical studies, since they are strongly associated in pathologies such as neurodegenerative and metabolic diseases [1,2].

Glycation, also known as the Maillard reaction, is a slow non-enzymatic reaction that initially involves attachment of glucose or derivatives with free amine groups of protein to form reversibly a Schiff base product, leading to the formation of stable fructosamine residue (ketoamine) following Amadori rearrangement [3]. Further oxidation and rearrangement of these early stage glycation products irreversibly give rise to advanced glycated end products (AGE) [4]. The glycation process is often associated with oxidative phenomenon, called "glycoxidation", which occurs when oxidative reactions affect the early stage glycation products [5].

Protein glycation and aggregation phenomenon are closely linked since common structural and functional properties are shared by amyloid aggregate type proteins and glycated proteins. Main cellular receptors for AGEs, including RAGE, CD36, MSR-A and MSR-B, are thought to be also primary transporters of amyloid type peptides [6,7]. Besides, protein glycation process could induce the formation of several types of aggregates such as globular forms or amyloid fibrils [8–10]. Terminal glycation products are well known to be closely involved in neurodegenerative diseases by promoting amyloid deposit in brain [11]. In addition, protein aggregates can impair insulin secretion in type II diabetes mellitus and indirectly promote glycation process of main circulatory proteins [12].

In these pathologies, metal ions (mainly copper, iron and zinc) are found to be highly concentrated within the core and periphery of

Abbreviations: AFM, atomic force microscopy; AGE, advanced glycated end product; BSA, bovine serum albumin; IncBSA, bovine serum albumin incubated at 37°C; MES, 2-(N-morpholino) ethane sulfonic acid; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; FTIR, Fourier Transform InfraRed; ThT, Thioflavin-T

* Correspondence to: G. Navarra, DiFC Università di Palermo, Via Archirafi 36, 90123 Palermo, Italy. Fax: +39 091 6162461.
** Correspondence to: P. Rondeau, GEICO, 15 Av René Cassin, France. Fax: +262 262 93 82 37.
E-mail addresses: giovanna.navarra@unipa.it (G. Navarra), rophil@univ-reunion.fr (P. Rondeau).

[1] These two authors contributed equally to this work and should be considered co-first authors.

0304-4165/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.12.017

amyloid senile plaque and also in the cortical tissue of the brain [13,14]. Besides, high amount of metal ions can be related to diabetes mellitus and neurological disorders [15]. If the role of these ions has not been fully clarified in vivo, several evidences suggest an inhibitor or promoting action of these metal ions in the aggregation process depending on the metal/protein ratio and the metal-ion binding mode. Previous studies from our group showed a differential effect of Cu(II) and Zn(II) ions in promoting bovine serum albumin or β-lactoglobulin aggregation [16,17].

Metal ions may be present during protein glycation process and metal-catalyzed oxidation reactions have several intersections with post-translational protein modification induced by glucose. Metal ion-mediated glucose autoxidation leads to the formation of high reactive carbonyl compounds [18]. Other studies reported a promoting role of metal ions (ferric Fe^{3+} and cupric Cu^{2+}) in α-oxoaldehyde formation from early glycation products or Amadori products [19,20]. In organisms, redox-active metals are transported by proteins. Among proteins which could sequester metal in non-reactive forms, human serum albumin represents the main target protein for glycoxidation. This plasma protein could serve as a circulating depot for many drugs and also metal ions [21,22]. In parallel, albumin is sustainably and continually exposed to oxidative stresses impairing its beneficial properties [23].

Glycation of serum albumin has been widely studied in recent years and the bovine form of albumin (BSA) constitutes the most common molecular model used in glycation research field. Even if albumin is well-known to bind various cationic metals, no data are available on the effect of these metals on albumin glycation. In order to clarify the potential roles of metal on albumin glycation, we focused our study on two metal ions: copper (II) and zinc (II). The glycation process of albumin in the presence or absence of metal was monitored by using biophysical (infrared and fluorescence spectroscopy, AFM microscopy) and biochemical (colorimetric and enzymatic assays) techniques. The impact of metal ions on albumin functionality and cellular cytotoxicity was also investigated.

2. Materials and methods

2.1. Materials

Non-recombinant bovine serum albumin (BSA) (cat# A9647), $CuCl_2$, $ZnCl_2$ and D-(+)-Glucose were from Sigma. AGE (BSAG samples) were prepared as previously described [24] by incubating filtered solutions of 0.4 mM BSA and 100 mM of glucose in MES (4-morpholineethanesulfonic acid) buffer (pH 7.2), under sterile conditions and nitrogen gas in capped vials at 37 °C for seven weeks. During this preparation, solution of 1 mM or 3 mM of copper ($CuCl_2$) or zinc ($ZnCl_2$) ions could be added. The proteins were dialyzed against MES buffer, pH 7.2 and stored at −20 °C. For FTIR absorption measurements, samples were washed with MES, pH 7.2, prepared in D_2O before the measurements.

2.2. Absorption and fluorescence measurements

Samples were diluted 1:60 with buffer (6.6 μM BSA). UV/Vis absorption measurements were carried out on UV-2401PC Shimadzu spectrophotometer to monitor protein concentration at 280 nm. The calculated molar absorptivity for bovine serum albumin is 46,824 $M^{-1}.cm^{-1}$ at 278 nm. Glycophore absorbance was measured at 340 nm. Spectra were corrected for scattering contribution, and normalized to metal ion absorption and to protein concentration.

Tryptophan, glycophore and Thioflavin-T fluorescence emissions were measured on our preparations (Jasco FP-6500). The tryptophan emission spectra were determined in the 280–500 nm range (270 nm excitation). AGE-related modifications were determined by measuring glycophore fluorescence at 425 nm under excitation at 345 nm. Fluorescence emission of Thioflavin-T was determined at 500 nm with an excitation at 430 nm.

2.3. FTIR absorption measurements

IR spectra were recorded by a Bruker Vertex 70 spectrometer, equipped with a MIR global light source (i.e. U-shaped silicon carbide piece). The spectral resolution was 2 cm^{-1} and the total number of scans for each spectrum was 100. All samples were placed between two CaF_2 windows, with a 0.05 mm Teflon spacer. The investigated infrared zone is the region of the Amide I. Amide I band is due to an out of phase combination of the C═O and C – N stretching modes of amide groups. D_2O solutions were used in BSA samples to avoid the spectral overlaps between Amide I band and strong absorption band of water at 1640 cm^{-1}. The main component in the Amide I region of BSA infrared absorption is located at about 1650 cm^{-1} and it is assigned to α-helices, the component at about 1680 cm^{-1} is assigned to β-turns, and the component at 1635 cm^{-1} is assigned to intra-molecular β-sheets.

2.4. Atomic force microscopy

Small aliquot (30 μl) of protein solution was loaded on freshly cleaved mica. The samples were dried overnight under nitrogen flux and imaged. The instrument used for the AFM measurements in tapping mode was a Veeco MultiMode V Scanning Probe Microscope. Etched-silicon probes with Al-coating on detector side having a pyramidal-shape tip with a nominal curvature <10 nm were used. During scanning, the 125 ±10 μm long cantilevers, with a nominal spring constant in the range of 40 N/m, oscillated at its resonance frequency (330 kHz). Height, phase and amplitude error images were collected by capturing 512 × 512 points in each scan, and the scan rate was maintained below 1 line per second. The worsening of the tips was monitored by using a test pattern before and after every measurement session.

2.5. Biochemical characterizations

Fructosamine was determined using the method developed by Johnson et al. [25] with the nitroblue tetrazolium (NBT) reagent and following a protocol described in a previous study [26]. The results are expressed as mmol/l of 1-deoxy-1-morpholinofructose (DMF), which is a synthetic ketoamine used as a primary standard.

TNBS (2, 4, 6-trinitrobenzenesulfonic acid) assay is a sensitive method for determining the primary free amino groups in proteins [27] and was described in a previous study by our group [28]. Various concentrations of L-glycine (10 to 200 nmol) were used as standards.

Thiol groups in native, modified albumin were measured by Ellman's assay using 5, 5′-dithiobis, 2-nitrobenzoic acid (DTNB) [29] as described previously [24]. A standard curve was performed for each assay and used various concentrations of L-cysteine (10 to 100 nmol) (Sigma). The content of thiol groups for each BSA sample was measured in duplicate on two different quantities of BSA by reading the absorbance at 412 nm. Results were expressed as the number of free – SH groups per mol of BSA. Aggregative modifications of glycated albumin samples in the presence or absence of metal were analyzed by native and SDS-Polyacrylamide Gels (12% of acrylamide) and stained by Coomassie blue according to Laemmli's method [30]. Level of carbonylation of proteins was determined by spectrophotometric assay based on recognition of protein-bound DNPH in carbonylated proteins with an anti-DNP antibody [31]. 200 μg of protein lysates was precipitated and suspended with 0.5 ml of DNPH solution (0.2% in 2 M HCl). Samples were incubated at room temperature for 10 min and precipitated in 10% TCA solution before centrifugation (2000 *g* for 2 min). Extraction of free DNPH from suspended pellets was performed with 3 successive washes with ethanol–ethyl acetate solution (50:50). Then dried protein pellets were dissolved in a 200 μl guanidine solution (6 M in 500 mM KCl pH 2.5). The absorbance