International Journal of Biological Macromolecules 93 (2016) 805–813

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Glyoxal administration induces formation of high molecular weight aggregates of hemoglobin exhibiting amyloidal nature in experimental rats: An in vivo study

Sauradipta Banerjee, Abhay Sankar Chakraborti *

Department of Biophysics, Molecular Biology & Bioinformatics, University of Calcutta, 92, Acharyya Prafulla Chandra Road, Kolkata 700009, India

ARTICLE INFO

Article history:
Received 24 July 2016
Accepted 16 September 2016
Available online 16 September 2016

Keywords:
Glyoxal
Hemoglobin
Advanced glycation end products
Mass spectrometry
Hydroimidazolone
Amyloid-like aggregation

ABSTRACT

Glyoxal, a highly reactive α-oxoaldehyde, increases in diabetic condition and reacts with proteins to form advanced glycation end products (AGEs). In the present study, we have investigated the effect of glyoxal on experimental rat hemoglobin in vivo after external administration of the α-dicarbonyl compound in animals. Gel electrophoretic profile of hemolysate collected from glyoxal-treated rats (32 mg/kg body wt. dose) after one week exhibited the presence of some high molecular weight protein bands that were found to be absent for control, untreated rats. Mass spectrometric and absorption studies indicated that the bands represented hemoglobin. Further studies revealed that the fraction exhibited the presence of intermolecular cross β-sheet structure. Thus glyoxal administration induces formation of high molecular weight aggregates of hemoglobin with amyloid characteristics in rats. Aggregated hemoglobin fraction was found to exhibit higher stability compared to glyoxal-untreated hemoglobin. As evident from mass spectrometric studies, glyoxal was found to modify Arg-30β and Arg-31α of rat hemoglobin to hydroimidazolone adducts. The modifications thus appear to induce amyloid-like aggregation of hemoglobin in rats. Considering the increased level of glyoxal in diabetes mellitus as well as its high reactivity, the above findings may be physiologically significant.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Post-translational modifications of proteins play important roles in controlling their functions. Reducing sugars react with amino groups of proteins, a process known as non-enzymatic glycation (Maillard reaction) resulting in browning, fluorescence and cross-linking of proteins [1]. There action consists of several steps, including Schiff's base formation, Amadori rearrangement etc. finally leading to formation of advanced glycation end products (AGEs). Formation of AGEs in vivo contributes to pathophysiologies associated with aging and complications of diabetes [2], which may be of quite significance due to increased levels of several active carbonyl compounds. Besides glucose, other glycating agents include fructose [3], glyoxal [4], methylglyoxal [5], 3-deoxyglucosone [6] etc. Findings from our laboratory indicate that glycation of hemoglobin by glucose [7,8], fructose [9] and methylglyoxal [10] promote iron release and free radical-mediated oxidative reactions.

The reactive α-oxoaldehydes namely, glyoxal, methylglyoxal and 3-deoxyglucosone are known to initiate Maillard-like reactions, and are more reactive than the parent hexose sugars with respect to their ability of protein modification and AGE formation [11,12]. Methylglyoxal is mainly derived from triose phosphates D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate during glycolysis in eukaryotic cells, and its blood level increases in both type 1 and type 2 diabetes mellitus [13–15]. It has been reported to react with several proteins namely, hemoglobin, cytochrome c, ceruloplasmin, myoglobin, etc., resulting in either protein cross-linking and aggregation or formation of non cross-linking AGE adducts (namely, hydroimidazolones, argpyrimidine, carboxyethyllysine, etc.) [10,16–19].

Like methylglyoxal, glyoxal is another reactive oxoaldehyde, and its concentration increases from 215 to 230 nM in normal individuals to 350–470 nM in diabetic subjects [15]. It is a major product of glucose degradation under oxidative conditions [20] and has been reported to interact with several proteins, namely, α-crystallin [21], bovine serum albumin [22], α-synuclein [23]

* Corresponding author.
E-mail addresses: ascbmbg@caluniv.ac.in (S. Banerjee), abhay_chakraborti@yahoo.co.in (A.S. Chakraborti).

http://dx.doi.org/10.1016/j.ijbiomac.2016.09.061
0141-8130/© 2016 Elsevier B.V. All rights reserved.

and hemoglobin [4,24]. Glyoxal modifies predominantly lysine and arginine residues of proteins to form several products, such as carboxymethyllysine [25], carboxymethylarginine [26], dihydroxyimidazolidines and hydroimidazolones.

In recent years several in vitro studies on the reactive α-oxoaldehydes, glyoxal and methylglyoxal have been reported. Besides in vitro studies, in vivo studies have also been carried out to investigate the effect of the reactive oxoaldehydes on experimental animal model. Methylglyoxal administration has been reported to induce diabetes-like complications, retinal injury as well as several other complications in experimental rats in earlier studies [27–31]. Glyoxal treatment has reported to cause renal damage in experimental rats in recent studies [32,33].

In recent studies, we have reported the effect of methylglyoxal and glyoxal on the heme proteins, hemoglobin and myoglobin in vitro [9,19,24,34]. In the present study, we have carried out animal experiments to investigate the effect of glyoxal on rat hemoglobin in vivo after administration of the reactive α-oxoaldehyde in animals. Characterization of glyoxal-treated rat hemoglobin has been carried out with respect to structure and stability changes, AGE modification(s) and aggregate formation.

2. Materials and methods

2.1. Materials

Glyoxal, Sephadex G-100, acrylamide, ThioflavinT (ThT), Coomassie R250, sequencing grade trypsin, α-cyano-hydroxycinnamic acid matrix (CHCA), were purchased from Sigma Chemical Company, USA. All other reagents were AR grade and purchased locally.

2.2. Methods

Animal experiments were performed in accordance with regulations specified and monitored by the Institutional Ethics Committee. Male Wister rats (weighing 70–80 gm) were divided in four groups – I, II, III and IV, consisting of four rats in each. Group I rats were not treated with glyoxal and denoted as control group. Groups II, III and IV rats were respectively injected with 8, 16 and 32 mg/kg body weight glyoxal intravenously and were denoted as glyoxal-treated group. All animals were fed with standard diet and water ad libitum. Whole blood was collected from each group after one week by heart puncture and hemolysates prepared.

2.2.1. Separation of hemoglobin and other fractions by size-exclusion column chromatography

Hemolysates prepared from different groups of rats were subjected to native PAGE. Total hemoglobin was purified from hemolysates of group I and group IV rats using Sephadex G-100column chromatography [35]. For group IV rats, fractions eluting before hemoglobin were also purified by column chromatography. A particular fraction exhibiting the presence of only high molecular bands in native PAGE (denoted as fraction 12 purified from hemolysate) was used for further characterization. The concentration of hemoglobin was determined from Soret absorbance using an extinction coefficient ($\varepsilon_{415\,nm}$) of 125 $mM^{-1}\,cm^{-1}$ (heme basis) [36].

2.2.2. MALDI-TOF mass spectrometric study

Purified hemoglobin obtained from control group I and glyoxal-treated group IV rats were subjected to mass spectral analysis using the linear positive ion mode of MALDI-TOF MS after digestion with sequencing-grade trypsin in solution at 37 °C for 16 h using enzyme: protein ratio 1:100 (w/w). For group IV rat hemolysate as well as fraction 12, desired bands of interest were excised from native gel and subjected to in gel trypsin digestion followed by mass spectral analysis. The digested samples (0.5 μl each) were loaded directly to the MALDI plate, mixed with 0.5 μl of saturated CHCA solution (prepared in 50% acetonitrile and 0.1% trifluoroacetic acid) and allowed to dry and crystallize. Mass spectra were recorded in a 4800 Proteomics Analyzer (MALDI-TOF/TOF mass spectrometer, Applied Biosystems) using the linear positive ion mode of MALDI-TOF MS at 20 kV acceleration voltage. Identification of glyoxal-modified peptides and specific glyoxal-derived AGE adducts was performed as described earlier [24]. Control group I rat hemoglobin and fraction 12 (purified from group IV rat hemolysate) were used for further experiments.

2.2.3. Absorbance spectroscopy

Absorbance spectrum of control rat hemoglobin sample (3 μM protein concentration) was recorded in a UV/VIS Spectrophotometer (Hitachi U 2000) using 1 ml quartz cuvette of path length 1 cm in the region 250–600 nm. Absorbance spectrum of fraction 12 was also recorded in the same region taking aliquot directly from the sample.

2.2.4. Fluorescence study

Fluorescence emission spectra of the samples were recorded in the region 320–400 nm with excitation at 280 nm in a spectrofluorimeter (Hitachi F-3010). Protein concentration was adjusted to 3 μM for recording each spectrum.

2.2.5. CD study

CD spectra of protein samples (3 μM each) were recorded in the far UV region (190–250 nm) in a spectropolarimeter (Jasco 600). The α-helical contents were calculated following the method of Chen et al. [37]. The percentage of β-sheet was determined by using the online web server K2D3.

2.2.6. ThT fluorescence microscopy

The samples (5 μl each) were directly mixed with 10 μl ThT (1 mM stock solution) and incubated at 37 °C for 30 min. The incubated samples were placed on glass slides and fluorescence was obtained after blue excitation (430 nm) of ThT-treated samples using appropriate filter. Images were acquired using an Olympus fluorescence microscope (BX 51) equipped with a digital CCD camera and Image-Pro Express software. The photographs were taken at 50× magnification.

2.2.7. Confocal microscopy

Confocal laser imaging of samples was done in a confocal microscope (LSM 510 Meta) equipped with a 405 nm diode laser as the excitation source and 40× objective. Emission between 450 and 500 nm was recorded.

2.2.8. XRD study

Samples were air-dried on glass slides and subjected to XRD analysis in a Rigaku X-ray powder diffractometer equipped with Cu anode (Cu-Kα radiation λ = 1.54186 Å). Scanning was done from 0° to 60° at 30 kV.

2.2.9. Stability studies

DSC study was carried out to measure thermal stability of samples. The melting profiles were recorded in a VP-DSC Microcalorimeter by heating the samples (1 °C/min) over a definite temperature range. Before introduction into the calorimetric cells, the protein samples were thoroughly degassed.

Conformational stability of samples was measured by chemical denaturant-induced unfolding study. Fluorescence emission spectra (300–400 nm) of samples (3 μM each) were recorded with

Download English Version:

https://daneshyari.com/en/article/6481704

Download Persian Version:

https://daneshyari.com/article/6481704

Daneshyari.com