Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 130 (2014) 561–567

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Hemoglobin fructation promotes heme degradation through the generation of endogenous reactive oxygen species

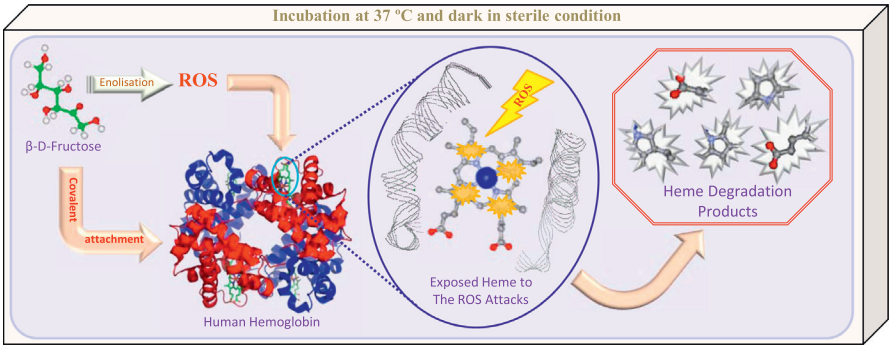
M. Goodarzi [a], A.A. Moosavi-Movahedi [a,b,*], M. Habibi-Rezaei [c,d], M. Shourian [a], H. Ghourchian [a], F. Ahmad [e], M. Farhadi [f], A.A. Saboury [a,b], N. Sheibani [g]

[a] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[b] Center of Excellence in Biothermodynamics, University of Tehran, Tehran, Iran
[c] School of Biology, University of Tehran, Tehran, Iran
[d] Center of Excellence in NanoBioMedicine, University of Tehran, Tehran, Iran
[e] Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi 110025, India
[f] ENT-HNS Research Center, IUMS, Tehran, Iran
[g] Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

HIGHLIGHTS

- Measuring ROS and heme degradation products by Hb incubation with fructose.
- Higher detected ROS concentration in fructose solution than fructated Hb solution.
- ROS amounts had an ascending trend during the 1st week and was not zero at time zero.
- Heme exposed to the solvent and heme degradation products began to accumulate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 25 March 2014
Accepted 4 April 2014
Available online 20 April 2014

Keywords:
ROS
Hemoglobin (Hb)
Chemiluminescence
Fructose
Heme degradation
Fructation

ABSTRACT

Protein glycation is a cascade of nonenzymatic reactions between reducing sugars and amino groups of proteins. It is referred to as fructation when the reducing monosaccharide is fructose. Some potential mechanisms have been suggested for the generation of reactive oxygen species (ROS) by protein glycation reactions in the presence of glucose. In this state, glucose autoxidation, ketoamine, and oxidative advance glycation end products (AGEs) formation are considered as major sources of ROS and perhaps heme degradation during hemoglobin glycation. However, whether fructose mediated glycation produces ROS and heme degradation is unknown. Here we report that ROS (H_2O_2) production occurred during hemoglobin fructation *in vitro* using chemiluminescence methods. The enhanced heme exposure and degradation were determined using UV–Vis and fluorescence spectrophotometry. Following accumulation of ROS, heme degradation products were accumulated reaching a plateau along with the detected ROS. Thus, fructose may make a significant contribution to the production of ROS, glycation of proteins, and heme degradation during diabetes.

* Corresponding author at: Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. Tel.: +98 21 66403957; fax: +98 21 66404680.
E-mail address: moosavi@ut.ac.ir (A.A. Moosavi-Movahedi).

http://dx.doi.org/10.1016/j.saa.2014.04.056
1386-1425/

Introduction

The Glycation or nonenzymatic reaction of the reducing sugars with free amino groups of proteins was described for the first time by Louis Camil Maillard in 1912. This modification begins with the reaction of the carbonyl group (aldehyde or ketone) of the sugar with the amino group of proteins to form a reversible Schiff base. The Schiff base can undergo intra-molecular rearrangements to form Amadori products in the early stages, and irreversible advanced glycation end products (AGEs) in the late stages of the complex cascade of reactions [1].

In spite of the fact that glucose is the main primary sugar contributing to glycation reactions, these reactions with other monosaccharaides may occur with higher stringency [2–4]. Fructation (fructose contribution to glycation) is a kind of bypass in glucation (glucose contribution to glycation) process [2]. In both (glucose by an aldehyde group and fructose by a *keton* group), the initial step is the covalent attachment between a free carbonyl group of open-chain carbohydrate and ε-amino group of protein to produce the Schiff base which is unstable. The Schiff base resulted from glucose undergoes Amadori rearrangement to produce Amadori products and the Schiff base resulted from fructose undergoes Heyns rearrangement to produce Heyns products [5,6]. Both Heyns compounds and Amadori products are derived from "early glycation products" or "fructosamines" [5,7]. Then enolization, dehydration, oxidation, and/or fragmentation of the Heyns products lead to produce a variety of ROS and other reactive compounds [6].

Moreover, due to higher intra-molecular stress and as a result higher population of open ring state, fructose is much more reactive than glucose toward glycation [2,8,9] and produce greater amounts of ROS and RCS [5,6,10–15].

In spite of fructose higher reactivity, its contribution to extracellular glycation has been less noteworthy than glucose. This is mainly attributed to the low plasma concentration of fructose compared with glucose. Intracellularly, exposure to hyperglycemia results in elevation of fructose in numerous cells and tissues in which the aldose reductase pathway is active. In these cells (such as red blood cells; RBCs) the activation of polyol pathway (which converts glucose to fructose), an important pathway in the development of diabetes complications, results in fructose accumulation reaching concentrations that are comparable to glucose [3,16]. Moreover, the dietary fructose is passively transported into the RBCs by the high-affinity fructose transporter GLUT5 as a member of the facilitative glucose transporter (GLUT) family and a component of nonhepatic clearance of dietary fructose [17,18]. As a result, a fourfold accumulation of fructose has been reported in the RBCs of diabetic patients disposing hemoglobin (Hb) to fructation [10]. That's why fructose is an important parameter in glycation studies because of its directness dependence to glycation process which is mediated by glucose.

Ketoaldehydes like fructose and α-hydroxyaldehydes such as glucose, which are prone to oxidation via their enediol rearrangements, can generate H_2O_2 and reactive intermediates including hydroxyl radicals. These chemical characteristics of monosaccharides and glycation of macromolecules could participate in various modifications during experimental conditions [19]. In this state, α,β-dicarbonyl compounds, produced by oxidative and/or non-oxidative pathways, may play a role as protein cross-linker in glycated proteins and alter their structures and functions [20–23].

The extent of protein structural and/or conformational changes is proportional to the hydroxyl radicals produced by glucose/fructose autoxidation and/or other related processes [6,19,23]. Free radicals originate by glucose/fructose autoxidation, the initial reaction of monosaccharides in the presence of proteins to form ketamine and ketoaminomethylol [24], and oxidative degradation of fructosamines in later stages to form AGEs [22]. These free radicals are present when biological macromolecules are exposed to high levels of monosaccharides [19]. However, the reaction of the open ring fraction of the sugar as an initialization and autoxidation intermediate [19,24,25], with protein amino groups may also retard the formation of self-produced H_2O_2 by the sugar [19].

In fructation, several pathways are involved to produce H_2O_2 including Wolff pathway (by monosaccharide autoxidation), Hodge pathway (by autoxidation of the Heyns compounds), and Namiki pathway (by Schiff base Oxidative fragmentation) in which reactive di/tri-carbonyls (RCS) and ROS (including H_2O_2) are produced [6,19,26–32]. Wolff and coworkers reported the generation of nanomolar levels of hydrogen peroxide in a mixture of bovine serum albumin (BSA) and glucose [25]. In spite of the low steady-state concentrations, H_2O_2 level is in the potential range for causing noticeable damage in proteins [19,25,33].

The heme group plays a vital role in the function of hemoglobin, and its surrounding environment dictates its function [34]. In the native protein, ferrous (Fe^{2+}) is coordinated with porphyrin and proximal histidine [35].

On one hand, there was a report for heme susceptibility to glucose because of its free iron capacity in non physiologic conditions [36]. On the other hand the production of two fluorescent degradation species during the reaction of oxyhemoglobin (not methemoglobin) with H_2O_2 have been reported which are distinct from enzymatic heme degradation products as bilirubin and biliverdin [37]. In this work, we tried to make a link between these two lines of researches by investigating the probability of ROS production during Hb fructation (using fructose as a glycation agent) with directly ROS measurements, monitoring the pattern of accumulation of heme degradation products, and comparing ROS production manner with heme degradation pattern. The aim is to become closer to the mechanism of heme degradation induced by fructation. So, here the production of ROS and heme degradation were studied upon incubation of hemoglobin with fructose. It was noticeable that heme degradation products, followed endogenous ROS generations, which were different from enzymatic heme degradation products. The study of heme degradation will give us new insight into the pathological impact of hyperglycemia and the development of effective and precise treatment for diabetic patients. Thus, the accumulation of these unknown products may have additional health risks in hyperglycemic patients.

Materials and methods

Materials

Fructose was obtained from Sigma (St. Louis, MO, USA). Luminol and other chemicals were purchased from Merck (Germany). All other reagents were of analytical grade.

Methods

Preparation of hemoglobin

Hemoglobin (Hb) was prepared from fresh blood collected from a healthy young person [38]. In brief, to prevent coagulation, sodium citrate (4%) in 1:9 volume ratios were added followed by separation of RBCs by centrifuging. RBCs were washed with 0.9% saline three times, then with phosphate buffer (200 mm, pH 7.4). The Lysis of RBCs was achieved by adding cold double distilled water to release hub Hb followed by debris precipitation. The final