International Journal of Biological Macromolecules 79 (2015) 336–343

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Inhibitory effect of quercetin in the formation of advance glycation end products of human serum albumin: An *in vitro* and molecular interaction study

Md. Maroof Alam, Irshad Ahmad, Imrana Naseem *

Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University Aligarh, India

ARTICLE INFO

Article history:
Received 30 November 2014
Received in revised form 4 May 2015
Accepted 6 May 2015
Available online 14 May 2015

Keywords:
Quercetin
AGEs
Lipid and protein oxidation
Comet assay
Molecular docking

ABSTRACT

Non-enzymatic glycation entails the reaction between the carbonyl group of a sugar with the amino group of a protein giving rise to Schiff base and Amadori products. The formation of advanced glycation end products (AGEs) leads to the generation of free radicals, which play an important role in the pathophysiology of ageing and diabetes. Bioavailable dietary antioxidants like quercetin (QC) are thought to inhibit AGEs formation. This study was aimed to investigate the effect of quercetin on AGE formation and features the glycation of human serum albumin (HSA) and its characterization by various spectroscopic techniques. The effect of quercetin, against the formation of AGEs was studied using a glycated human serum albumin product, haemoglobin-δ-gluconolactone, and aminoguanidine. The results were then corroborated with estimation of protein oxidation, lipid peroxidation and comet assay. On the basis of the experimental data, computational docking studies were then performed to understand the location of the site of quercetin binding and its best bound conformation with respect to human serum albumin. Through this study we have demonstrated the mechanism of formation of AGE and its inhibition by quercetin. We have also suggested that the supplementation with dietary antioxidants like quercetin might protect against free radical toxicity.

1. Introduction

Glycation is the non-enzymatic reaction initiated by a nucleophilic addition reaction of free reducing sugars with free amino groups of DNA, proteins and lipids. The glycation starts with the formation of highly unstable Schiff base, which undergoes a rearrangement to form an Amadori product [1]. Amadori product further undergoes a series of reactions through dicarbonyl intermediates and generate cross-linked and fluorescent derivative to form advance glycation end products (AGEs). The major AGEs in vivo appear to be formed from highly reactive intermediate carbonyl groups, known as oxoaldehydes or -dicarbonyls, including 3-deoxyglucosone, methylglyoxal and glyoxal [2,3]. Some of the best chemically characterized AGEs in humans include pentosidine and *N*-ε-carboxymethyllysine (CML). Apart from endogenously formed products, AGEs can also originate from exogenous sources such as tobacco smoke and diet [4,5]. Food processing, especially prolonged heating, has an accelerating effect in the generation of glyco-oxidation and lipo-oxidation products, and a significant proportion of ingested AGEs is absorbed with food. Tissue and circulating AGEs level are higher in smokers and in patients on high AGE diets, with concurrent increase in inflammatory markers [6]. With age AGEs accumulate in vascular wall tissues and on plasma lipoproteins and bind to AGE specific receptors (RAGEs). AGEs bind to RAGE at an accelerated rate in diabetic patients and implicated various problem because they can alter enzyme activity, modify protein half-life, decrease ligand binding and alter immunogenicity [7–10]. AGEs binds to RAGE on smooth muscle cells increases the chemotactic migration and cellular proliferation which leads to over-production of reactive oxygen species and over-generation of reactive carbonyl species, production of proinflammatory cytokines in individuals and high restenosis rates in patients with diabetes and renal failure [11,12].

Many studies have indicated that methylglyoxal (MG) forms AGEs by reacting with DNA, proteins and lipoproteins endogenously in enzymatic and non-enzymatic reactions under physiological conditions [13,14]. A number of studies with human subjects have shown that diet-derived AGEs precursors such

* Corresponding author at: Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, U.P. 202002, India.
Tel.: +91 571 2700741/91 9719069125; fax: +91 571 2706002.
E-mail address: imrananaseem2009@gmail.com (I. Naseem).

http://dx.doi.org/10.1016/j.ijbiomac.2015.05.004
0141-8130/

as *N*-ε-carboxymethyllysine (CML) and methylglyoxal (MG) are bioavailable and may enhance inflammatory responses and oxidative stress in individuals with debilitating diseases, such as diabetes [7,15]. Exposer to MG- or AGE-rich foods over a prolonged period of time may result in ROS formation with corresponding degradative changes in different tissues. Thus, CML and MG are glycation intermediates and precursor of AGEs, and relevant targets for compounds aimed at reducing the undesirable consequences of protein glycation both in vitro and in vivo. The glycation of proteins with sugar have been extensively reported and occur mainly via lysine and arginine residues of human serum albumin (HSA) and immunoglobulin-G (IgG) [16,17]. It is well reported that guanine residue of DNA primarily involved in glycation with glucose [18]. The phosphate form of dihydoxyacetone i.e., dihydroxyacetone phosphate takes part in glycolysis, contribute to rapid formation of AGEs and caused DNA damage [19,20]. Thus, further study needed in context of DHA adduct formation and severity of glycation at the early, intermediate and advanced stages of the reaction.

It is well established that dietary bioactive compounds, like curcumin, ellagic acid, flavonoids, EGCG or thymoquinone inhibit AGEs formation in vitro and in vivo [21,22]. Quercetin (QC) is one of the most abundant of all flavanoids and is found in fruits, vegetables, leaves, grains and red grape wine [23]. It can be used as an ingredient in supplements, beverages or foods. Many reports have shown that quercetin has high affinity towards human serum albumin. Furthermore, the interaction study of HSA with antioxidant polyphenols has a biological importance [24]. Quercetin is also a well-known quencher for reactive oxygen species generated by any physical or chemical action [25]. There is growing interest in natural products with combined anti-glycation and antioxidant properties as they may have reduced toxicity. Indeed, a number of plant derived polyphenol with antioxidant activity have been reported to inhibit glycation. Previously we have reported that quercetin counteracts oxidative stress generated in type 2 diabetes mellitus [26]. Thus, we have extended our work to explore the effect of quercetin on AGEs formation.

2. Materials and methods

2.1. Chemicals and materials

Human serum albumin (HSA), aminoguanidine (AG), methylglyoxal (MG), gluconolactone, dihydroxyacetone (DHA), quercetin, Histopaque 1077, RPMI 1640 were purchased from Sigma–Aldrich Chemical Company, USA. Fresh human blood samples were taken in citrate dextrose preparations. DMSO, glucose, DTNB were obtained from SRL chemicals (India). Quercetin was dissolved in dimethyl sulfoxide (DMSO) to a stock solution of 25 mM. All other reagents were of analytical grade.

2.2. Human serum albumin (HSA) in vitro glycation assay

Human serum albumin (20 mg/ml) was simultaneously incubated with 60 mg/ml of glucose in 20 mM PBS (pH 7.4) containing 0.02% NaN_3 at 37 °C for 25 days under aseptic conditions in the presence or absence of quercetin at various concentration. The negative control contained HSA and the test compound but without glucose. Aminoguanidine (10 mM) was used as a positive control and incubated with HSA in the presence of 60 mg/ml glucose. After incubation, samples were extensively dialyzed against PBS in order to remove excess of sugars and stored at −20 °C for further analysis. Protein concentration was measured by Lowry method [27].

2.3. Spectroscopic analysis

The ultraviolet absorption spectra of native and glycated HSA samples were recorded in the wavelength range 200–400 nm on Shimazdu UV-1700 PharmaSpec UV–vis spectrophotometer.

2.4. Fluorescence analysis

Fluorescence emission profile of native and glycated HSA were recorded on Shimazdu RF-5301 spectrofluorometer. Both native and glycated HSA were excited at 370 nm and emission profile was recorded.

2.5. Circular dichroism (CD) measurements

CD spectra were recorded on JASCO spectropolarimeter (J-815) calibrated with D-10-camphorsulfonic acid. The measurements were made at 25 °C with a thermostatically controlled cell holder attached to Neslab's RTE 110 water bath with a temperature accuracy of ±0.1 °C. Far-UV CD was used to measure the changes in the secondary structure of HSA in 20 mM phosphate buffer (pH 7.4). Protein samples were placed in cylindrical quartz cuvettes of path length 1 mm. Each spectrum was the result of average of four scans [28].

2.6. Haemoglobin-δ-gluconolactone (δ-Glu) reactivity

The haemoglobin-δ-gluconolactone assay was performed according to Losso protocol with minor modification [29]. Fresh human blood (400 μl) was mixed with 80 μl of 0.2 M PBS (pH 7.4) as the blank. Fresh blood (400 μl) was also mixed with 80 μl of 0.2 M PBS (pH 7.4) and 50 mM of δ-Glu as the control. Test samples contained 400 μl of blood, 80 μl of 0.2 M PBS containing 50 mM of δ-Glu and 25–200 μM of quercetin. Similarly, aminoguanidine (10 mM, final concentration) was incubated with 400 μl of blood and 50 mM of δ-Glu as positive control. After 20 days incubation at 37 °C, the presence or absence of glycated haemoglobin (HbA1c) was determined. All the blood samples were analyzed in triplicates. The percent inhibition of HbA1c formation by the compound was calculated by $(B-C)/(B-A) \times 100$, where A is HbA1c concentration in the base line control tube not treated with δ-Glu, B is the HbA1c content of the test tube treated with δ-Glu, and C is the HbA1c levels in the sample treated with both δ-Glu and the compound.

2.7. Methylglyoxal-HSA reactivity

The method of Lee was followed with some minor modification [30]. Briefly, HSA (50 mg/ml) was incubated with 40 mM methylglyoxal (MG) in the absence or presence of 25, 50,100 and 200 μM quercetin under sterile conditions in 20 mM phosphate buffer (pH 7.4) at 37 °C for 14 days.

$$\text{The \% inhibition of AGEs formation} = [1 - (\text{fluorescence of the test group/fluorescence of the control group})] \times 100$$

2.8. Isolation of lymphocytes

Heparinised blood sample (4 ml) from a single healthy donor was obtained by venepunture and diluted suitably in Ca^{2+} and Mg^{2+} free PBS. Lymphocytes were isolated from blood using Histopaque 1077 and cells were finally suspended in RPMI 1640. The